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In re the application of
MASAHIRO IMOTO et. al.

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For: SUBSTITUTED 1-AZA-2-IMINOHETEROCYCLES AND THEIR USE AS
NICOTINIC ACETYLCHOLIN RECEPTORS ACTIVATORS

DECLARATION

I, Yoshihiro Tani, Ph.D., a citizen of Japan residing at 25-9, Tamasecho, Ibaraki-shi, Osaka, 567-0893, Japan, declare as follows.

1. I graduated from Faculty of Pharmaceutical Sciences, Osaka University of Pharmaceutical Sciences in 1984.
2. I graduated from Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University in 1986.
3. I entered Suntory Limited as a researcher at the Suntory Institute for Biomedical Research in 1987, and have been assigned as a senior researcher of pharmaceutical research laboratories since 1994.
4. I obtained the Ph.D. in 1991 from Kyushu University.
5. I worked as a researcher at Uppsala University PET center, Sweden from 1991 to 1992.

The following is my opinion regarding the inventions of this application based on my own knowledge applying the technical background.

Neuronal nicotinic receptor agonists have attracted much interest as potential therapeutic agents for the treatment of cognitive impairments associated with Alzheimer's disease, schizophrenia and Parkinson's disease. Clinical studies have revealed that (-)-nicotine is effective to ameliorate memory and attention deficits in Alzheimer's disease patients (Newhouse et al 1986; Sahakian et al 1989; Jones et al 1992). In animals, (-)-nicotine has been reported to show beneficial effects on memory in aged monkeys and to reverse spatial memory deficits in rat with an experimental

lesion of the medial septal nucleus (Levin 1992; Decker et al 1995). In addition, the centrally acting nicotinic receptor channel blocker, mecamylamine, produces significant cognitive impairment that mimics certain aspects of Alzheimer's disease in young and elderly volunteers (Newhouse et al 1994). Postmortem studies of Alzheimer's disease brain tissue demonstrated marked reductions of nicotinic receptors in both neocortex and hippocampus, consistent with the Alzheimer's disease pathology of neuronal degeneration (Araujo et al 1988). These findings point to the functional importance of nicotinic acetylcholine systems in cognitive functions (for recent reviews, see Rezvani and Levin 2001; Newhouse et al 2001).

Regarding the several types of neuronal nicotinic receptor ligands recently discovered, extensive pharmacological and behavioral studies have been carried out on (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT-418) (Garvey et al 1994), selective agonist at $\alpha 4\beta 2$ subunits more than $\alpha 3$ and $\alpha 7$ subunit of neuronal nicotinic receptor (Arneric et al 1994). ABT-418 showed potent cognition-enhancing properties in improving retention of avoidance learning in normal mice and attenuated lesion-induced deficits in a spatial memory in animal model of Alzheimer's disease (Decker et al 1994a; Decker et al 1994b). ABT-418 was the first novel selective nicotinic agonist tested in human patients. In placebo-controlled design study, ABT-418 showed significant dose-related improvement in learning and memory in early to moderate Alzheimer's disease patients (Potter et al 1999). However, ABT-418 is no longer in development by Abbott due to lack of oral bioavailability and the separation between the dosages for the therapeutic effect and the dosages for the potential cardiovascular side effects were too small to be acceptable.

Clinical studies indicate that (-)-nicotine may be beneficial for the treatment of impairment in attention and rapid information processing associated with Alzheimer's disease, and imply that not only the cholinergic system but also monoaminergic systems are possible mechanisms by which (-)-nicotine treatment improves cognitive performance. Among the monoaminergic systems, it has been suggested that noradrenergic effects of stimulants as important therapeutic mechanisms on enhancing capabilities such as attention and working memory.

Therefore, after performing the binding assays at two types of nicotinic receptors and agonist activities at human $\alpha 4\beta 2$ subunits of nicotinic receptors using *Xenopus* oocytes, we evaluated the effects of the compounds of the present invention on norepinephrine (NE) turnover in the mouse whole brain as the first *in vivo* assay. It has been reported that brain NE turnover is enhanced by systemic administration of (-)-nicotine in various brain regions and its effect is blocked by the centrally acting nicotinic receptor channel

blocker, mecamylamine (Morgan and Pfeil 1979; Kubo et al 1989). The effects of nicotinic receptor agonists such as (-)-nicotine and ABT-418 were also evaluated as reference compounds.

Materials and methods

Male ddY mice (6 weeks of age, Nihon SLC, Shizuoka, Japan) were used. They were housed in climate-controlled room (room temperature $23 \pm 1^{\circ}\text{C}$ and humidity $55 \pm 5\%$) and allowed free access to food and water. Mice were killed by decapitation, after 30 min the compound or drug was administered subcutaneously (s.c.). The mouse whole brain was homogenated in 2.0 ml of 0.1 M perchloric acid containing 0.1 % $\text{Na}_2\text{S}_2\text{O}_5$ and 0.1 % EDTA2Na, followed by the addition of 3,4-dihydrobenzylamine (DHBA) 50 ng as the internal standard. After centrifugation at 1000 g for 20 min, the supernatant was stored at -80°C until assay. The concentrations of NE and 3-methoxy-4-hydroxy-phenylglycol (MHPG), the NE metabolite, were determined by use of a liquid chromatography (LC) system with electrochemical detection. The LC system consisted of a PM-60 pump (BAS) set at 1.2 ml/min, connected to a reverse-phase column (Cosmosil 5C18, 250 mm x 4.6 mm i.d., $5\mu\text{m}$) maintained at 35°C with a column heater (LC-22A, BAS). NE and MHPG were detected with an electrochemical detector (LC-4B, BAS) set at a potential 750 mV versus the Ag/AgCl reference electrode. The mobile phase was 0.1 M sodium acetate/citric acid buffer, pH 4.80 containing 8 % methanol and 4.6 mM sodium 1-octanesulfonate. The results were statistically analyzed using the Dunnett's two-tailed multiple comparison test. A probability level of $p < 0.05$ was considered significant.

Results and Discussion

The dose-response studies of nicotinic agonists ((-)-nicotine and ABT-418), and 6 compounds (No. 7, 11, 14, 23, 27 and 29) of the present invention on NE turnover in the whole brain of mice were performed (Table 1). (-)-Nicotine increased both MHPG content and MHPG/NE ratio in a dose-dependent manner, and showed significant increases at doses of 1.0 and 5.0 mg/kg. The selective agonist at $\alpha 4\beta 2$ subunits, ABT-418 showed enhancement of both MHPG content and MHPG/NE ratio in a dose-dependent manner, but significant increase was observed at the highest dose of 5.0 mg/kg. The compounds of the present invention also showed significant effects on both MHPG content and MHPG/NE ratio. The compound No.7 and 23 induced significant

increase in NE turnover in a dose-dependent manner, and minimum effective dose was 5.0 mg/kg. The compound No.11, 14, 27 and 29 also exhibited significantly increases of MHPG/NE ratio at 10.0 mg/kg.

Table 1 Effects of (-)-nicotine, ABT-418 and the compounds of the present invention on NE turnover in the mouse whole brain.

Compound	Dose	MHPG	NE	MHPG/NE
	(mg/kg s.c.)	(% of saline group)		
No.7	1.0	105.1±5.1	103.7±1.3	100.8±4.9
	5.0	121.0±9.3	95.8±2.8	126.2±9.3 *
	10.0	133.8±6.2 *	100.4±3.1	133.0±4.9 *
No.11	10.0	116.3±6.8	96.9±2.6	120.1±6.0 *
No.14	10.0	113.0±5.2	95.6±3.1	118.7±5.3 *
No.23	1.0	116.3±4.2	106.2±3.2	109.5±3.9
	5.0	126.3±6.7 *	107.3±5.5	118.2±5.6
	25.0	178.1±10.2 *	106.2±2.5	168.6±13.1 *
No.27	10.0	111.9±4.7	94.2±1.9	119.0±5.2 *
No.29	10.0	112.3±6.8	93.1±2.5	120.0±4.0 *
(-)-nicotine	0.04	106.0±3.7	99.4±3.1	107.1±5.4
	0.2	107.0±6.6	101.8±4.2	105.0±5.9
	1.0	143.5±0.4 *	100.2±4.5	142.3±2.2 *
	5.0	226.9±19.9 *	93.1±3.1	240.5±14.1 *
ABT-418	0.2	104.1±2.6	101.0±3.3	103.3±2.3
	1.0	109.8±4.7	95.9±2.0	114.3±3.7
	5.0	160.1±5.7 *	104.0±2.8	154.6±7.0 *

Animals were killed 30 min after the compound or drug administration. Values in the Table are expressed as percent change from control levels 30 min after saline treatment. * p<0.05; significantly different from saline group (Dunnett's two-tailed test, mean ± SEM, n=7-8).

Among novel nicotinic receptor agonists, ABT-418 showed significant improvement both in experimentally induced animal models (Decker et al 1994b) and in early to moderate Alzheimer's disease patients (Potter et al 1999). ABT-418 was a selective agonist at $\alpha 4\beta 2$ subunits of nicotinic receptor, since ABT-418 had high affinity for [3 H]cytisine binding ($K_i = 3$ nM) but was inactive in 37 other receptor,

neurotransmitter-uptake, enzyme, transduction system binding assays, and ABT-418 was equipotent to (-)-nicotine in stimulating [$^{86}\text{Rb}^+$] efflux from mouse thalamus that was thought to reflect the activation of $\alpha 4\beta 2$ subunits of nicotinic receptor (Arneric et al 1994). We also confirmed such abilities of ABT-418 using receptor binding assays and *Xenopus* oocytes expressing $\alpha 4\beta 2$ subunits of nicotinic receptor.

Regarding the mechanisms by which (-)-nicotine enhanced brain NE turnover, previous our studies have indicated that (-)-nicotine enhances brain NE turnover may be attributed to activation of $\alpha 4\beta 2$ subunits but not $\alpha 7$ nicotinic receptors (Tani et al 2002). Because, (-)-nicotine-induced increase in NE turnover was blocked dose-dependently by pretreatment with dihydro- β -erythroidine (DH β E), a competitive nicotinic receptor antagonist that reported to be more sensitive to $\alpha 4\beta 2$ subunits, but a selective nACh-R antagonist for $\alpha 7$ subunit, methyllycaconitine (MLA) did not affect (-)-nicotine-induced increase in NE turnover. The neuronal nicotinic receptors are thought to be composed of α and β subunit and the most abundant nicotinic receptor in the central nervous system consists of $\alpha 4$ and $\beta 2$ subunits (Flores et al 1992), while in recombinant expression system $\alpha 7$ subunit can form functional homooligomeric receptor. What subunits of nicotinic receptors might mediate cognition-enhancing properties is as yet unclear, but $\alpha 4\beta 2$ subunits of nicotinic receptor appear to have the greatest relevancy to Alzheimer's disease and other cognitive disorders (Newhouse et al 2001).

The six compounds No. 7, 11, 14, 23, 27 and 29 of the present invention had high affinity for [^3H]cytisine binding. Studies with *Xenopus* oocytes expressing $\alpha 4\beta 2$ subunits of nicotinic receptor also demonstrated that these compounds had the abilities as agonists for $\alpha 4\beta 2$ subunits of nicotinic receptor. The present study indicated that a single systemic administration of these compound significantly enhanced brain NE turnover, and the relative potencies for enhancement of brain NE turnover were (-)-nicotine > ABT-418 > compound No.7 and No.23 > compound No.11, 14, 27 and 29. Therefore, these findings suggest that the six compounds No. 7, 11, 14, 23, 27 and 29 of the present invention may exhibit cognition-enhancing properties in both animal model of Alzheimer's disease and patients with Alzheimer's disease.

References

- Araujo, D.M., Lapchak, P.A., Robitaille, Y., Gauthier, S., Quirion, R., 1988. Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. *J. Neurochem.* 50, 1914-1923.

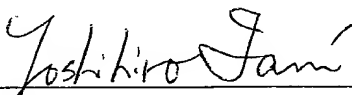
- Arneric, G.W., Sullivan, J.P., Briggs, C.A., Donnelly-Roberts, D., Anderson, D.J., Raszkiewicz, J.L., Huges, M.L., Cadman, E.D., Adams, P., Garvey, D.S., Wasicak, J.T., Williams, M., 1994. (S)-3-Methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT-418): A novel cholinergic ligand with cognition-enhancing and anxiolytic activities: I. In vitro characterization. *J. Pharm. Exp. Ther.* 270, 310-318. **
- Decker, M.W., Brioni, J.D., Sullivan, J.P., Buckley, M.J., Radek, R.J., Raszkiewicz, J.L., Kang, L.H., Kim, D.J.B., Giardina, W.J., Wasicak, J.T., Garvey, D.S., Williams, M., Arneric, S.P., 1994a. (S)-3-Methyl-5-(1-methyl-2-pyrrolidinyl)-isoxazole (ABT-418): A novel cholinergic ligand with cognition-enhancing and anxiolytic activities: II. In vivo characterization. *J. Pharm. Exp. Ther.* 270, 319-328. **
- Decker, M.W., Curzon, P., Brioni, J.D., Arneric, S.P., 1994b, Effects of ABT-418, a novel cholinergic channel ligand, on place learning in septal-lesioned rats. *Eur. J. Pharmacol.* 261, 217-222.
- Decker, M.W., Brioni, J.D., Bannon, A.W., Arneric, S.P., 1995. Diversity of neuronal nicotinic acetylcholine receptors: lessons from behavior and implications for CNS therapeutics. *Life Sci.* 56, 545-570.
- Flores, C.M., Rogers, S.W., Pabreza, Z.A., Wolfe, B.B., Kellar, K.J., 1992. A subtype of nicotinic cholinergic receptor in rat brain is composed of alpha 4 and beta 2 subunits and is upregulated by chronic nicotine treatment. *Mol. Pharmacol.* 41, 31-37.
- Gravey, D.S., Wasicak, J., Decker, M.W., Brioni, J.D., Sullivan, J.P., Carrera, G.M., Holladay, M.H., Arneric, S.P., Williams, M., 1994. Novel isoxazoles which interact with brain cholinergic channel receptors that have intrinsic cognitive enhancing and anxiolytic activities. *J. Med. Chem.* 37, 1055-1059.
- Jones, G.M.M., Sahakian, B.J., Levy, R., Warburton, D.M., Gray, J.A., 1992. Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. *Psychopharmacology* 108, 485-494.
- Kubo, T., Amano, H., Kurahashi, K., Misu, Y., 1989. Nicotine-induced regional changes in brain noradrenaline and dopamine turnover in rats. *J. Pharmacobiodyn.* 12, 107-112.
- Levin, E.D., 1992. Nicotinic systems and cognitive function. *Psychopharmacology* 108, 417-431.
- Morgan, W.W., Pfeil, K.A., 1979. Mecamylamine blockade of nicotine enhanced noradrenaline turnover in rat brain. *Life Sci.* 24, 417-420.

- Newhouse, P.A., Sunderland, T., Thompson, K., Tariot, P.N., Weingartner, H., Mueller, E.R., Cohen, R.M., Murphy, D.L., 1986. Intravenous nicotine in patient with Alzheimer's disease. *Am. J. Psychiatry* 143 1494-1495.
- Newhouse, P.A., Potter, A., Corwin, J., Lenox, R., 1994. Age-related effects of the nicotine antagonist mecamylamine on cognition and behavior. *Neuropsychopharmacology* 10, 93-107.
- Newhouse, P.A., Potter, A., Kelton, M., Corwin, J., 2001. Nicotinic treatment of Alzheimer's disease. *Biol. Psychiatry* 49, 268-278. **
- Potter, A., Corwin, J., Lang, J., 1999. Acute effects of the selective cholinergic channel activator (nicotinic agonist) ABT-418 in Alzheimer's disease. *Psychopharmacology* 136, 50-58. **
- Rezvani, A.H., Levin, E.D., 2001. Cognitive effects of nicotine. *Biol. Psychiatry* 49, 25-267.
- Sahakian, B.J., Jones, G., Levy, R., Gray, J.A., Warburton, D.M., 1989. The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of Alzheimer's type. *Br. J. Psychiatry* 154, 797-800.
- Tani, Y., Oka, T., Ohno, T., 2002. Pharmacological characterization of nicotinic acetylcholine receptor (nACh-R)-mediated changes in brain norepinephrine (NE) turnover in mice. *Jpn. J. Pharmacol.* 88, suppl. 189P.

**Note: The four references underlined are attached herein.

I, the undersigned petitioner, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 28th day of November, 2003



Yoshihiro Tani, Ph.D.

This 28th day of November, 2003

Toshio Tatsuoka

Witnessed by Toshio Tatsuoka, Ph.D.
General Manager

Nicotinic Treatment of Alzheimer's Disease

Paul A. Newhouse, Alexandra Potter, Megan Kelton, and June Corwin

Approximately 20 years after the formulation of the "cholinergic hypothesis" to explain the cognitive symptoms of Alzheimer's disease, cholinesterase therapy remains the mainstay of treatment for this disorder. Although partially effective, currently available agents have limited effects on cognitive function and long-term efficacy appears modest. Direct or indirect stimulation of nicotinic cholinergic receptors may offer an additional therapeutic strategy. Ongoing investigations of the molecular substructure of central nervous system nicotinic receptors, their accompanying pharmacology, and the effects of nicotinic agents on cognitive function have suggested the possibility that nicotinic stimulation may have beneficial effects in Alzheimer's disease and other neuropsychiatric disorders. Studies from our laboratory and others have explored the role of central nervous system nicotinic mechanisms in normal human cognitive and behavioral functioning as well as their role in Alzheimer's disease. Results from acute therapeutic trials with nicotine and novel nicotinic agents suggest that nicotinic stimulation in Alzheimer's disease patients can improve the acquisition and retention of verbal and visual information and decrease errors in cognitive tasks, as well as improve accuracy and response time. Whether such results will translate into improved clinical functioning remains to be fully tested. Development of subtype-selective nicotinic agonists with an improved safety profile will enable long-term testing of the efficacy of nicotinic stimulation on cognitive performance as well as potential cytoprotective effects. Direct or indirect (allosteric) modulation of nicotinic receptor function offers a new opportunity for Alzheimer's disease therapeutics. Biol Psychiatry 2001;49:268-278 © 2001 Society of Biological Psychiatry

Key Words: Alzheimer's disease, nicotine, nicotinic agonists, mecamylamine, ABT-418, treatment

From Clinical Neuroscience Research Unit, Department of Psychiatry, University of Vermont College of Medicine, Burlington (PAN, AP, MK), the Department of Psychology, Dartmouth College, Hanover, New Hampshire (MK), and Psychiatry Service, Manhattan Veterans Administration Medical Center, New York University School of Medicine, New York, New York (JC).

Address reprint requests to Paul A. Newhouse, M.D., University of Vermont College of Medicine, University Health Center, Department of Psychiatry, 1 South Prospect Street, Burlington VT 05401-1195.

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Introduction

The underlying cause(s) of Alzheimer's disease (AD) appear to be disruption of the regulation, expression, or scavenging of abnormal membrane-associated proteins (e.g., β -amyloid leading to neurotoxicity). This neurotoxicity leads to a degeneration of a variety of neurotransmitter systems that presumably are responsible for the clinical and cognitive manifestations of the illness. Although a myriad of neurochemical deficits have been described in AD, explanation of the nature of the cognitive disturbances has been most closely focused on the "cholinergic hypothesis," which implicates disturbances in central muscarinic cholinergic mechanisms in normal cognitive functioning and disorders of memory function (Bartus et al 1982; Drachman and Leavitt 1974).

Nicotinic mechanisms may be important in explaining the pathophysiology and in designing treatments for AD (James and Nordberg 1995). Patients suffering from AD have a marked reduction in cortical nicotinic cholinergic receptor binding relative to age-matched control subjects (Aubert et al 1992; Flynn and Mash 1986; Whitehouse et al 1986). Normal aged subjects show an age-related decline in cortical nicotinic binding (Flynn and Mash 1986). Warpman and Nordberg (1995) used the nicotinic agonists epibatidine and ABT-418 to show selective losses of $\alpha 4\beta 2$ nicotinic receptors in the brains of patients with AD. Perry et al (1995) showed that the entorhinal cortex (important in memory formation), rich in nicotinic binding, appears particularly vulnerable to amyloid plaque-induced loss of receptors. More generally, Perry and colleagues (Perry et al 1995) have shown that nicotine receptor loss seems tightly linked to the primary pathology in the dementias (e.g., linked to dopaminergic cell loss in Parkinson's disease [PD] and Lewy Body dementia) and linked to amyloid plaques and tangles in hippocampal and parahippocampal areas in AD.

Neuroimaging studies also support the involvement of nicotinic cholinergic systems in AD. Nordberg (1993) showed a significant correlation change between the change in temporal cortex labeling of [^{11}C]nicotine and cognitive function scores in AD patients using positron emission tomography (PET). This result was bolstered by further work from these investigators (Nordberg et al 1995) in which a kinetic model was developed to quantify

the loss of nicotinic receptor binding *in vivo* in AD patients. Significant correlations were shown between cognitive dysfunction and the loss of nicotinic receptor binding in temporal and frontal cortices and the hippocampus in these patients using PET. Nordberg (1993) also examined the effects of treatment with the anticholinesterase tacrine on AD patients using PET and showed that brain nicotinic receptor binding of [^{11}C]nicotine increased along with cerebral blood flow after 3 weeks of treatment.

Interest in the possibility of utilizing agents that directly interact with nicotinic receptors for the treatment of central nervous system (CNS) disease has followed as understanding of the structure, function, and distribution of CNS nicotinic receptors has increased. Ongoing investigations of the molecular substructure of CNS nicotinic receptors and their pharmacology have begun to open up new possibilities for novel CNS therapeutics with nicotinic agents (Americ et al 1995). There is considerable evidence from both animal and human studies for the involvement of CNS nicotinic cholinergic receptors in a variety of cognitive, motor, and behavioral systems. Modulation of these receptors with the ultimate goal of producing therapeutic benefits is the goal of these investigations and drug development.

Research in this laboratory has focused over a number of years on studies designed to understand more fully the role of central nicotinic systems in normal and disordered human cognition, particularly degenerative neuropsychiatric conditions such as AD and PD. Furthermore, we have conducted pilot studies of nicotinic drugs as "proof of concept" studies to assist in the design of longer term clinical trials of potential nicotinic agents. We briefly review those studies here and interpret them in the light of renewed interest in nicotinic mechanisms in AD and PD and the potential for meaningful nicotinic modulation of cognitive and behavioral functioning.

Nicotinic Antagonist Studies

Studies utilizing antagonists are useful for establishing the cognitive relevance of neuroreceptor changes in the brain as they produce a temporary chemical "lesion." Newhouse and colleagues (Newhouse et al 1992, 1993, 1994) have studied the effects of the centrally active noncompetitive nicotinic antagonist and peripheral ganglionic blocker mecamylamine on cognitive functioning in young ($n = 12$, mean age 24) and elderly ($n = 15$, mean age 63) normal subjects and patients with mild to moderate AD ($n = 11$, Global Deterioration Scale = 3-5) and PD ($n = 11$, Hoehn-Yahr stages 1-2) disease. These studies showed that nicotinic blockade produced cognitive impairment in humans and that there were age- and disease-related changes in sensitivity to nicotinic blockade, indicated by

shifts in dose-response curves between groups. Mecamylamine administration produced dose-related impairment of the acquisition of new information with group differences in sensitivity. Young normal subjects showed significant cognitive impairment after the highest dose. By contrast, the elderly normal subjects showed significant impairment after the middle and high doses, and the AD subjects showed impairment after all three active doses.

The Repeated Acquisition Task, a nonverbal serial learning task, was used to show the effects of nicotinic blockade. Subjects are asked to learn a series of button pushes on a specialized keyboard with feedback provided only by the computer. As feedback is only provided by the computer and initial choices are random, it is possible to examine the shape of the learning curve and maintenance of learning over time. Furthermore, as a sequence of button pushes (chain) is taught before the drug administration begins, it is possible to examine both well-learned information (previously learned chain) and new learning that takes place after drug administration (new chain). In addition, the task is easily adapted to populations with different learning capacities simply by changing the length of the chain.

Mecamylamine produces errors in the acquisition phase of this task. Examination of individual curves shows that mecamylamine impaired both the acquisition of new information and the ability to hold that information in working memory. Figure 1 demonstrates that, as subjects become older and/or demented, the sensitivity to the error-producing effects of mecamylamine increases, demonstrated by a shift in the dose-response curve to the left. However, results from the "performance" phase of this task (not shown) demonstrate no dose-related impairment, suggesting that nicotinic blockade does not affect the retrieval of well-learned information.

Negative effects of nicotinic blockade can also be seen in the acquisition of new verbal information using the Selective Reminding Task. Although there were no significant effects in young normal volunteers, mecamylamine produced a significant increase in recall failure at the highest dose of mecamylamine tested (20 mg) in elderly volunteers and impairment at all active doses in patients with AD. In the AD patients, the learning rate (amount of information learned or acquired divided by the amount of information remaining to be learned) actually became negative at 10 and 20 mg of mecamylamine (Figure 2), suggesting that subjects were forgetting recently learned information faster than they were acquiring new information.

In addition, mecamylamine produced a dose-related slowing of reaction time and liberalization of response bias in recognition memory tasks. This latter effect is particularly interesting, given that response bias measures

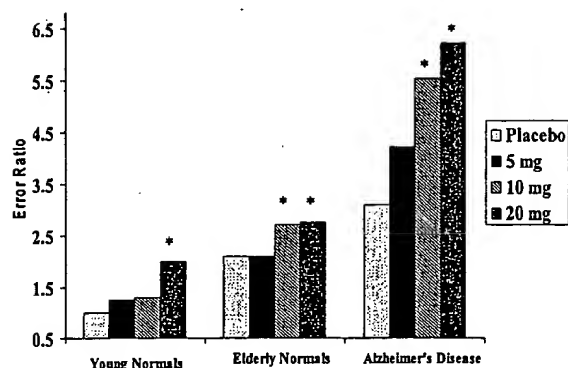


Figure 1. The effects of oral mecamylamine on performance of the learning phase of the Repeated Acquisition Task in younger normal subjects ($n = 12$, mean age 24), old normal subjects ($n = 15$, mean age 63), and Alzheimer's disease patients ($n = 11$, Global Deterioration Scale = 3–5). The error ratio is a calculation used to allow data from disparate groups to be presented in a single figure because the chain length required to be learned was different in normal volunteers and Alzheimer's disease patients. The error ratio was calculated by dividing the total number of errors over 20 trials by the length of the chain required to be learned for each subject. For normal volunteers the chain length was 10, and for Alzheimer's disease patients the chain length varied but was generally 4–6. * $p < .05$, different from the placebo.

are abnormal in AD. Response bias is a mathematical expression of decision-making criteria under conditions of uncertainty. Liberal response bias tends to be characteristic of AD patients and may be clinically manifest in the overinclusiveness and nonselectivity of AD patients' memory performance (Snodgrass and Corwin 1988). These results suggest that the deficits produced by mecamylamine resemble in several respects those seen in AD. Deficits in short- and long-term memory, impaired attention, liberal response bias, and decreases in reaction time are hallmarks of the dementing picture seen in these

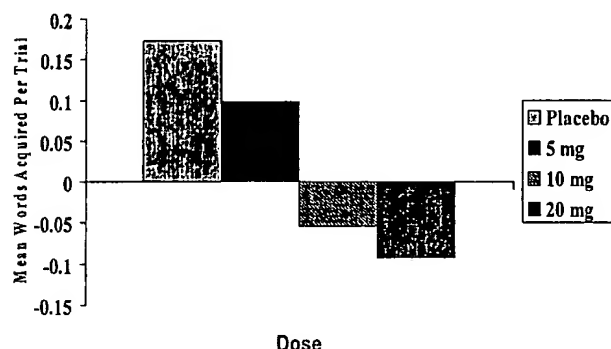


Figure 2. Learning rate (amount of information learned divided by amount remaining to be learned per trial) from the Selective Reminding Task in Alzheimer's disease patients ($n = 11$, Global Deterioration Scale = 3–5) after varying doses of mecamylamine.

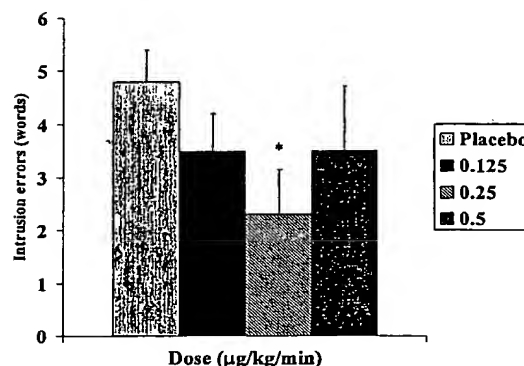


Figure 3. Effects of differing doses of intravenous nicotine on intrusion errors in a serial word learning task in subjects with Alzheimer's disease ($n = 6$, Global Deterioration Scale = 3–5). Infusion was 60 min in duration. * $p < .05$, different from the placebo.

disorders. The age- and disease-related nature of some of the findings supports the hypothesis that increasing sensitivity is related to the increasing loss of nicotinic receptors.

Although a full comparison is beyond the scope of this review, there are similarities and differences between the effects of the nicotinic antagonist mecamylamine and muscarinic antagonists, such as scopolamine or atropine, on cognitive function. Mecamylamine generally produces qualitatively similar though quantitatively smaller effects on acquisition of new information—for example, in the Repeated Acquisition Task (Higgins et al 1989) or verbal learning (Newhouse et al 1988; Sunderland et al 1989). The combination of muscarinic and nicotinic blockade produces additive effects, particularly on explicit memory performance (Little et al 1998). Mecamylamine does not generally produce significant effects on arousal, as is commonly seen with muscarinic antagonists (Robbins et al 1997), and effects on behavior are minimal relative to the effects of scopolamine (Newhouse et al 1994).

Nicotinic Agonist Effects

We have previously examined the effects of intravenous nicotine on cognitive, behavioral, and physiologic functioning in both normal nonsmokers and patients with AD (Newhouse et al 1988, 1993, 1996). After pilot studies aimed at establishing tolerable doses of intravenous nicotine for nonsmokers, we administered a series of intravenous doses over 60 min to a small group of patients with mild to moderate AD. The major cognitive effect seen was a significant dose-related reduction in intrusion errors on a serial learning free-recall task (Figure 3). Examination of the results shows a clear "U"-shaped dose-response curve with maximal improvement at 0.25 μg/kg/min of nicotine base.

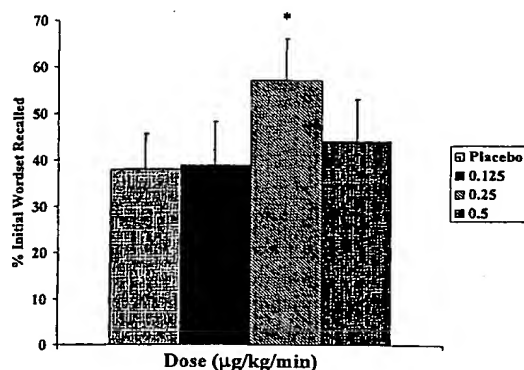


Figure 4. Effects of differing doses of intravenous nicotine on long-term (8 hours after infusion) recall in a serial word learning task in subjects with Alzheimer's disease ($n = 6$, Global Deterioration Scale = 3-5). Infusion was 60 min in duration. * $p < .05$, different from the placebo.

When subjects were retested for recall of the initial word set 8 hours after the drug infusion was completed, information that was initially recalled under the conditions of the 0.25-µg dose was significantly better retained (Figure 4), suggesting a memory consolidation effect of nicotine.

Neuroendocrine measures show that nicotine induced secretion of adrenocorticotrophic hormone and cortisol in a dose-related manner (Newhouse et al 1990) and tended to confirm that the doses used were active at CNS nicotinic receptors. In a later study we examined the effects of intravenous nicotine in AD with particular attention to tasks that are affected by mecamylamine (Newhouse et al 1996). Nicotine produced improvements in attentionally driven tasks, with improved reaction time, hits, and false alarms on a continuous performance task. Throughput (speed-accuracy product) was improved as well.

These findings of the beneficial results of acute nicotinic stimulation in AD have been supported by the studies of Sahakian and colleagues (Jones et al 1992; Sahakian and Coull 1994), who have shown that subcutaneous nicotine administration in AD patients produced improvements in attentional functioning. More chronic administration of nicotine to AD patients has also shown promise. Wilson and colleagues (Willson et al 1995) administered nicotine by patch to six AD patients for 8 days. Relative to the placebo patch condition, there were significantly fewer errors on the Repeated Acquisition Task while subjects were on nicotine. This effect persisted for at least a week after withdrawal. White and Levin (1999) studied 4 weeks of nicotine administration by transdermal patch in eight subjects with mild to moderate AD. The dose of nicotine varied from 5 to 10 mg/day across a 4-week period. Although no significant effects were seen on clinical measures, significant cognitive effects occurred, with im-

proved performance on the Continuous Performance Test with reduced errors (particularly errors of omission) and reduced Reaction Time variance. A composite attention measure combining speed and accuracy showed improvement over the course of nicotine administration, suggesting an increase in throughput or work performed per unit time. However, Snaedal and colleagues (Snaedal et al 1996) were unable to find a significant effect of 4 weeks of transdermal nicotine administration on memory in 18 AD patients, possibly due to a significant placebo effect, as patients on both nicotine and a placebo showed improvements in short-term memory.

The occurrence of side effects at higher doses, such as anxiety, adverse mood changes, and nausea/vomiting, and the steepness of the dose-response confirmed animal data suggesting that, although there was evidence for therapeutic potential for nicotine in AD, the therapeutic index of nicotine itself is small and the risk of adverse side effects is significant, at least for intravenous administration. This and other work led to a search for more selective nicotinic agonists that would have a better therapeutic index, improved pharmacokinetics, and higher degree of efficacy in AD patients. ABT-418 (Abbott Laboratories, Abbott Park, IL) was the first novel selective nicotinic agonist tested in human patients. Potter and colleagues (Potter et al 1999) studied six patients with early to moderate AD in a within-subjects, repeated-measures placebo-controlled design to examine acute effects of this novel agent on cognitive functioning.

Subjects were administered doses of 6, 12, and 23 mg via a transdermal device for 6 hours. When tested at the 6-hour time point, subjects showed significant linear dose-related improvements in verbal learning and memory on the Selective Reminding Task as reflected by improved total recall and a decline in recall failure. When the change in recall and recall failure on this task was examined across the 6-hour testing period, it could be seen that ABT-418 maintained or improved performance across the day in a dose-related way relative to placebo treatment, which allowed deterioration (Figure 5). This improvement was quantitatively similar to that seen in previous acute trials with anticholinesterase inhibitors. Qualitatively similar, though not statistically significant improvements were seen in nonverbal learning tasks such as spatial learning and memory and repeated acquisition. Positive dose-related effects (speeding) on reaction time were also seen. Interestingly, subjects also showed a decline in anxiety and fear self-ratings at the 23-mg dose, consistent with prior animal studies suggesting that this agent may also have anxiolytic effects (Brioni et al 1994). No adverse behavioral, vital sign, or physical side effects were seen. These positive results echo studies of this agent in aged monkeys by Buccafusco and colleagues (Buccafusco et al

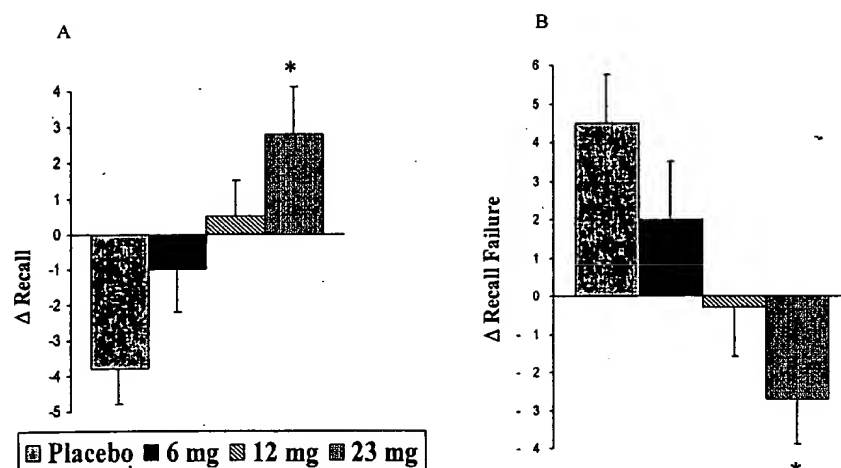


Figure 5. Effects of differing doses of the novel nicotinic agonist ABT-418 on performance of the Selective Reminding Task in subjects with Alzheimer's disease ($n = 6$, Global Deterioration Scale = 3-5). (A) Mean change in total words recalled (sum of five trials) from baseline to end of drug administration (6 hours). (B) Mean change in recall failure (defined as failure to recall a word after two successive reinforcements) from baseline to end of drug administration (6 hours). * $p < .05$, different from the placebo.

1995), who showed dose-related improvements in a delayed matching-to-sample task performance following administration of ABT-418.

Preclinical studies of other novel nicotinic agonists also show promise. Aged rats show improved learning when treated with the $\alpha 7$ selective agent GTS-21 (Taiho Pharmaceutical, Tokyo) (Arendash et al 1995). SIB-1553A (Merck, Whitehouse Station, NJ) is an $\alpha 4\beta 2$ subtype-selective nicotinic agonist and appears to be efficacious in acute and chronically stimulating hippocampal acetylcholine release (Lloyd et al 1998). This compound appears to produce enhanced performance in a variety of models of cognitive dysfunction (e.g., aged rats, rhesus monkeys, rats with cholinergic lesions) in areas such as spatial and nonspatial working and reference memory (Lloyd et al 1998). A profile such as this suggests that this compound may have activity in disorders of cortical and subcortical cholinergic dysfunction such as AD. RJR-2403 (Targacept, Winston-Salem, NC) appears to be a highly selective ligand for the $\alpha 4\beta 2$ subtype of nicotinic receptor and is the initial compound in a series of metanicotinic analogs to be explored for the treatment of degenerative neuropsychiatric disorders (Lipiello et al 1996). Selective nicotinic agonists may show a greater therapeutic index than nicotine itself, although they are unlikely to be totally free of nicotinelike adverse effects such as dizziness and nausea. Such side effects were reported in a clinical trial of ABT-418 in adult attention-deficit/hyperactivity disorder patients (Wilens et al 1998) and in trials of the novel nicotinic agonist SIB-1508Y for PD (McClure 1999).

Discussion

These studies represent significant evidence that stimulation of nicotinic receptors can improve the acquisition and retention of verbal (declarative) and nonverbal informa-

tion in humans. Previously, it has been difficult to demonstrate that stimulation of nicotinic receptors produces true learning or memory improvement in effects in normal subjects (Heishman et al 1994). The role of attentional effects of nicotinic stimulation has been stressed by Sahakian and Coull (1994). However, as has been suggested by Warburton and Rusted (1993), nicotine's effects are most often seen in tasks that have a large attentional load. For example, the verbal learning tasks that showed improvement in the AD patients after acute administration of ABT-418 required focused attention and significant cognitive effort. Two recent studies by Mancuso and colleagues (Mancuso et al 1999a, 1999b) have clarified the effects of nicotinic stimulation on attentional processes. In a study of the effects of nicotinic stimulation on rapid visual information processing, a relatively low dose of nicotine was shown to improve vigilance processing in young abstinent smokers, and the effect appeared to be concentration independent (Mancuso et al 1999a). In a second study (Mancuso et al 1999b), the investigators observed that nicotinic stimulation enhances the speed of processing and number generation. There were no effects on interference performance or attentional switching. The authors interpreted these findings as consistent with the possibility that nicotinic stimulation improves the intensity feature of attention rather than affecting the selectivity of attentional processes. In essence, this appears to suggest that stimulation of nicotinic cholinergic neurons provides more attentional resources in a nonspecific way rather than improving the ability of the attentional system to select information. This can also be interpreted as providing more processing resources, which may be of additional benefit to the cognitively impaired patient. Although it appears that nicotinic modulation can improve performance on tasks that require focused attention (either verbal or spatial) and are speed dependent, nicotinic

modulation may not improve performance on tasks that require access to semantic memory, retrieval of well-learned information, or switching between sources of information (e.g., between working memory domains).

It also may be the case that any cognitive-enhancing effects of nicotinic stimulation are more clearly manifest in cognitively impaired individuals. Nicotinic stimulation appears to show significant baseline dependency—that is, cognitive and performance effects appear to be determined in part by the baseline state of the individual (Perkins 1999). Therefore, subjects operating at a low level of performance will likely have their performance enhanced by nicotinic stimulation, whereas subjects operating at normal or above normal levels may have their performance diminished. This may help to explain why it has been difficult to demonstrate that nicotinic stimulation produces improvement in normal individuals except under conditions of deprivation. Conversely, it may be predicted that nicotinic stimulation may improve the performance of cognitively impaired individuals.

The studies reviewed here as an aggregate argue for the relevance of nicotinic receptor function in normal human cognition, motor, and possibly behavioral functioning as well as their role in disease states such as AD and PD. Though there are limitations to these studies (small sample size, wide variability in the effects of the principle agonist nicotine), they support further research into more fully elucidating nicotinic receptors' physiologic roles. Data from these and other studies regarding the effects of nicotinic stimulation and blockade allow a preliminary synthesis regarding the role(s) of these receptor systems in normal and disordered human cognitive functioning. The loss of or alterations to nicotinic receptors and/or their associated processes may be responsible for some of the cognitive changes and blood flow alterations that are seen in AD and other neuropsychiatric disorders. Nicotinic systems appear important to normal learning and memory, but effects may be in part mediated through effects on certain aspects of attentional functioning. Effects of nicotinic receptor activation may be mediated through presynaptic regulation of catecholaminergic, cholinergic, γ -aminobutyric acid (GABA)-ergic, and/or glutamatergic transmitter mechanisms in widespread projections to the prefrontal and/or parietal cortices and basal ganglia-thalamic-prefrontal loops. What subtypes of nicotinic receptors might mediate these effects is as yet unclear, although $\alpha 4\beta 2$ appears to have the greatest relevance to AD and other cognitive disorders. The role of the $\alpha 7$ receptor is less clear in AD, but stimulating this receptor may be of value as well, particularly for sensory processing and/or cytoprotection.

Data suggest that nicotinic systems and/or receptors are modulatory of the release of acetylcholine, dopamine,

norepinephrine, GABA, and other neurotransmitters onto their receptors. Therefore, there are probably limits to the actions of this system, and the loss of these receptors may result in the loss of a degree of control of cognitive processes rather than the underlying basic cognitive function itself. Certain cognitive processes affected in AD may not be under nicotinic modulation or influence. It appears more likely that nicotinic systems act to modulate or control the "front end" of memorial processing (e.g., control and partitioning of attentional resources that are critical to appropriate encoding of memories). Nicotinic modulation may also act to reduce the impact of distraction and allow more focused attention. For example, nicotinic stimulation may reduce distraction by improving sensory gating in an auditory physiology task (P50) in humans, possibly through $\alpha 7$ stimulation in the hippocampus (Adler et al 1992), or reduce the impact of distraction on the recall of visual information, perhaps through $\alpha 4\beta 2$ -induced catecholamine stimulation (Prendergast et al 1998).

Activation of nicotinic receptors in the visual cortex appears to promote intracolumnar inhibition and change the direction of information flow within cortical circuits, enhancing excitatory control of synaptic inputs to pyramidal cells (Xiang et al 1998). Such control, if generalized, may provide an additional mechanism whereby nicotinic stimulation may improve information processing.

These mechanisms may help to control the flow of information into and out of working memory, from the outside or from long-term store, inhibiting irrelevant and augmenting salient information. Alternatively, nicotinic modulation may increase information processing resources nonselectively, improving the performance of effort-demanding tasks, particularly under conditions of impaired functioning such as in AD. Although stimulation of this system is unlikely to restore full function, it may augment remaining cell connections, increasing information (signal) traffic, and therefore improve cognitive function. Preliminary evidence suggests that, although attentional effects can be manifested very rapidly with nicotinic agonists, significant learning and memory effects may need longer administration or exposure to nicotinic agonists than can be provided for by a short-lived single-dose administration.

Straightforward nicotinic receptor activation may not be the sole mechanism whereby nicotinic modulation may improve cognitive functioning. There is some evidence that, under certain conditions, desensitization or functional blockade of nicotinic receptors may enhance performance for certain types of cognitive operations. Evidence from preclinical studies in animals suggests that low doses of the $\alpha 4\beta 2$ -antagonist mecamylamine may actually enhance cognitive functioning under some circumstances (Driscoll

relevance (in) 相关性.

1976; Terry et al 1999). There are hints of this in human studies as well, especially when a nicotinic antagonist is given at low doses to relatively normal functioning individuals (Newhouse et al 1994; P.A. Newhouse et al, unpublished data). Further, there are data showing that the nicotinic antagonist mecamylamine may be as therapeutic as the nicotinic agonist nicotine for patients suffering from Tourette's syndrome (Sanberg et al 1998). Even the novel nicotinic agonist ABT-418, which we have shown produces therapeutic effects in patients with AD, may act as an antagonist under certain conditions (Papke et al 1997). A recent study by Fujii and colleagues (Fujii et al 2000) examined the modulatory effect of nicotine on the induction of long-term potentiation (LTP), a synaptic model of learning and memory. These investigators showed that nicotine was able to promote the induction of LTP, apparently by reversing inhibitory postsynaptic potentials produced in the presence of a GABA receptor agonist. Intriguingly, this effect was also seen with the $\alpha 7$ nicotinic antagonist methyllycaconitine, suggesting that nicotinic receptor inactivation or blockade may have been involved in producing the positive effect of LTP. The seeming paradox of both a nicotinic agonist and antagonist producing potentially therapeutic effects may be a specific example of the baseline dependency phenomenon in nicotinic pharmacology (Perkins 1999)—that is, it may be that cognitive and other performance-related operations function best at an optimal level of nicotinic stimulation and that reduction of nicotinic hyperactivity (e.g., Tourette's syndrome) by blockade or desensitization or an increase in hypoactivity (e.g., AD) by stimulation may be beneficial. Other useful agents may possess nicotinic antagonist properties as well—for example, the antidepressant bupropion, also used for smoking cessation, has been recently shown to possess nicotinic antagonist properties (Fryer and Lukas 1999). However, the use of a nicotinic antagonist such as mecamylamine for AD is less likely to be of benefit because of the left-shift in the dose-response curve for this agent (Newhouse et al 1994), presumably due to the disease-induced loss of nicotinic receptors.

Figure 6 presents a simplified general scheme for how nicotinic stimulation may, through influencing or modulating a broad range of neurotransmitter systems, improve the performance of attentional systems and therefore result in an improvement in learning and/or memory in AD. In this particular diagram, nicotinic-induced release or modulation of several different neurotransmitters is suggested, along with a particular receptor subtype that may be associated with that action. The effects of this transmitter release either separately or together may result in improvement in a number of different cognitive operations affected by, or under the modulatory control of, these

neurotransmitters. Generally, this scheme suggests that nicotinic stimulation affects attentional systems either by increasing the absolute activity of those systems or by improving discriminatory or inhibitory functioning (at this time it is not clear whether this increase in activity is due to stimulation or blockade/desensitization of nicotinic receptors). This may lead to improvements in working memory through simply increasing the total amount of attentional resources or more effective partitioning by the so-called central executive (Baddeley 1988), as well as direct improvements in psychomotor speed. The suggested anatomic loci for these effects are by no means exclusive nor are the connections suggested to be definitive. Nonetheless, evidence exists for nicotinic effects on all of these different cognitive operations and/or domains, and therefore such a diagram is a reasonable first step in attempting to synthesize an overall theory of how nicotinic stimulation influences cognitive performance. Note that this scheme pertains completely to the acquisition side of information processing. There is less information regarding nicotinic effects on retrieval of information.

The most likely direct therapeutic role for nicotinic agonists is as augmentation therapy in combination with other agents rather than as monotherapy, except early in disease states or as a prophylactic or preventative treatment. A major problem with nicotinic compounds relates to side effects. Can a compound be developed that is selective in producing improvement in cognition or attention without significant side effects (i.e., with an adequate therapeutic index)? The critical issue is whether a more receptor-specific compound with an improved risk/benefit ratio can be developed.

The treatment of AD has been and continues to be almost exclusively with agents that act predominantly to inhibit acetylcholinesterase. It has been presumed that their therapeutic efficacy is solely mediated by this action. Although partially effective, currently available cholinesterase inhibitors have significant limitations that preclude more than modest therapeutic efficacy. Dose-limiting side effects and nonspecific stimulation of all cholinergic synapses limit both cognitive effects and behavioral benefits (Weinstock 1999). Whether these agents are disease-modifying agents remains to be demonstrated. Other potential disease-modifying strategies have not demonstrated efficacy thus far as monotherapies—for example, antiinflammatories such as prednisone (Aisen et al 2000), COX-2 inhibitors (Rogers 2000), or hormones such as estrogen (Mulnard et al 2000). Etiologic strategies, even if they prove effective, are far from clinical implementation. Muscarinic agonists also have not shown efficacy thus far (Thal et al 2000). A new therapeutic strategy is justifiable and necessary.

Nicotinic receptor augmentation may be a worthwhile

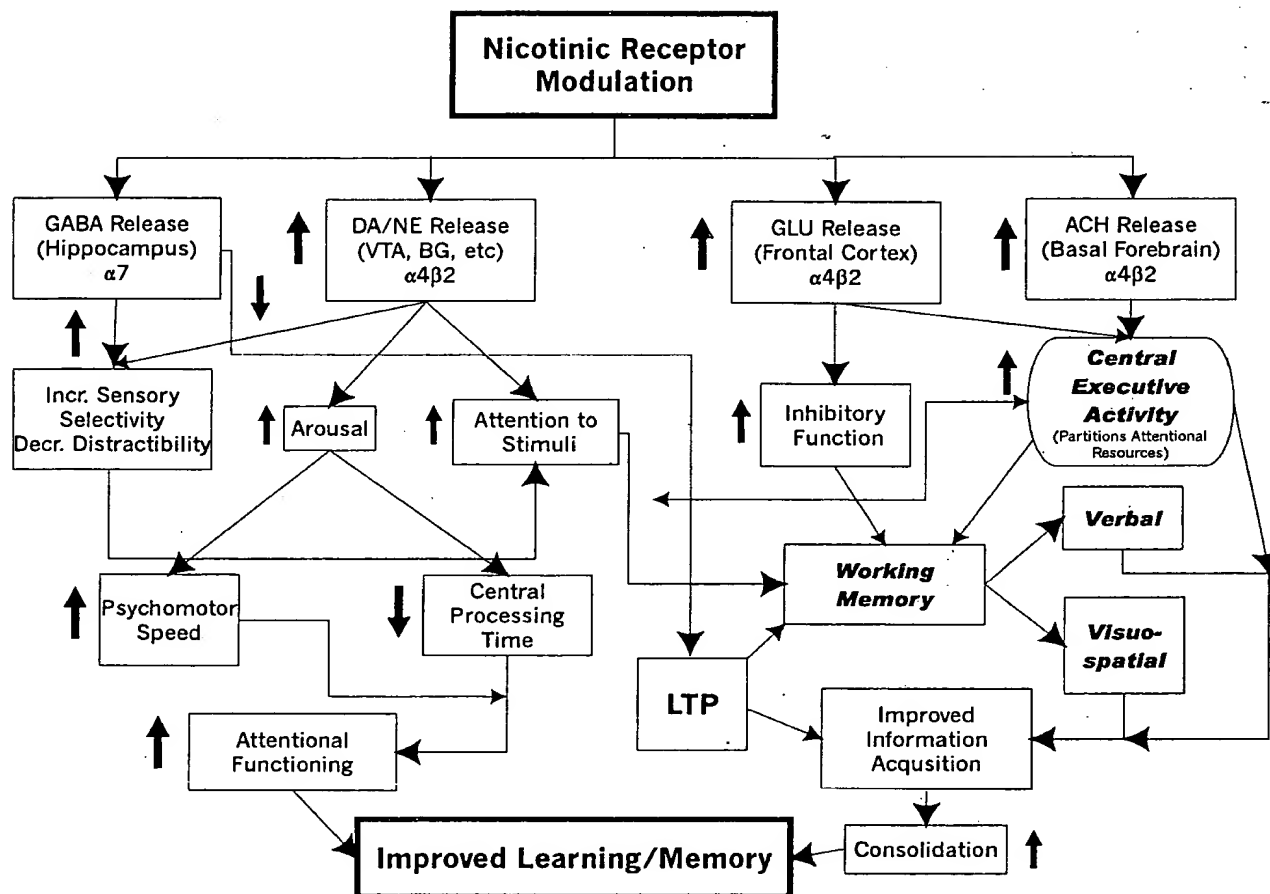


Figure 6. Simplified proposed scheme for nicotinic enhancement of attention, learning, and/or memory in degenerative disorders through actions on multiple neurotransmitters and associated cognitive operations. Data exist for potential nicotinic effects in all areas indicated in the figure (for further details, see Newhouse and Kelton 2000; Paterson and Nordberg 2000). A neuroanatomic location and potential mediating receptor subtype are suggested within each neurotransmitter box (not exclusive of other locations or subtypes). Arrows to the left of boxes indicate increased or decreased transmitter release or cognitive function. Bolded italicized components indicate elements of Baddeley's model of working memory. GABA, γ -aminobutyric acid; DA, dopamine; NE, norepinephrine; VTA, ventral tegmental area; BG, basal ganglia; GLU, glutamate; ACH, acetylcholine; LTP, long-term potentiation.

strategy to pursue for both symptomatic improvement and disease modification in AD (and perhaps PD as well). Nicotinic stimulation may produce a more rapid onset of cognitive improvement than currently existing cholinesterase inhibitors, as a result of the low threshold necessary for nicotinic receptor-induced phasic stimulation of pre-synaptic neurotransmitter-releasing receptors or through allosteric modulation of nicotinic receptor function simultaneously with cholinesterase inhibition (Maelicke and Albuquerque 2000). Whether this can be sustained and translates into noticeable clinical improvement remains to be seen. It may be possible to simultaneously directly and/or indirectly stimulate nicotinic receptors while inhibiting cholinesterase function through the use of agents that have simultaneous cholinesterase inhibition effects and

nicotinic allosteric effects (e.g., galantamine, physostigmine). This may lead to enhanced cholinergic activity in those regions activating cognitive operations that are cholinergically modulated. This may in turn increase information processing resources and allow greater throughput (useful cognitive work per unit time).

Potentially more rapid onset of effects with nicotinic agonists relative to cholinesterase inhibitors alone may be a result of the necessary pharmacokinetic and pharmacodynamic properties that a cholinesterase inhibitor must have to be tolerable (i.e., slow onset of cholinergic enhancement to avoid intolerable side effects due to nonspecific cholinergic stimulation). The fact that many patients do respond favorably, albeit slowly, to cholinesterase inhibitors suggests that the cholinergic system is

still plastic enough to respond to treatment. Nicotinic stimulation may be orthogonal or synergistic to cholinesterase inhibition rather than duplicative. In a recent pre-clinical study of animal learning, nicotinic agonist treatment has been shown to enhance the positive effects of cholinesterase inhibitors on cognitive performance (Bencherif 1999). This suggests it may be possible to enhance cholinergic functioning with nicotinic agents in combination with cholinesterase inhibitors to a greater degree than with cholinesterase inhibitors alone.

In addition to AD, changes in CNS cholinergic-nicotinic systems have also been shown to occur in the brains of patients with PD. In particular, a similar loss of cholinergic cells in the basal forebrain nuclei, as occurs in AD, has been described in PD (Whitehouse et al 1983). The loss of cholinergic markers in the cortex (Perry et al 1995) that occurs in PD may be related to lesions in these nuclei and other cholinergic projections to the cortex (Whitehouse et al 1988). Studies have shown a marked reduction in cortical nicotinic receptor binding that parallels the degree of dementia in PD and increasing age (Aubert et al 1992; Whitehouse et al 1988). There is similarity between the cortical nicotinic binding site loss in PD and AD as well as similar changes in other cholinergic markers.

We have recently examined the quantitative effects of nicotine in a pilot study in PD patients (Kelton et al 2000). Subjects showed positive effects on motor and cognitive performance both acutely and after 2-week transdermal nicotine administration. Further double-blind studies are necessary to confirm these results, but if they are, this would provide optimism that nicotinic stimulation may be an additional strategy for PD treatment, either by utilizing nicotinic agonists as monotherapy in early cases or as a dopa-augmenter or dopa-sparing drug in later stage disease.

The development of selective nicotinic agents for human use has made substantial progress in the last decade (Lloyd and Williams 2000). A variety of agents have been and are being tested in humans for a variety of indications including AD, PD, attention-deficit/hyperactivity disorder, schizophrenia, Tourette's syndrome, and pain. Whether long-term benefit can be obtained from these agents will be a test of both pharmaceutical development and clinical relevance. A potential alternate method for stimulating nicotinic receptor function is through allosteric modulation of sites on the receptor complex physically distinct from the agonist recognition site (Maelicke and Albuquerque 2000). Although enhancement of nicotinic receptor function by this mechanism utilizing cholinesterase inhibitors has been demonstrated in vitro (Maelicke and Albuquerque 2000), there is so far a lack of in vivo or human data suggesting these effects can be detected separately from the more direct effects of these agents.

If by direct stimulation or allosteric modulation such activity translates into increased nicotinic receptor activity, not only may more rapid or more extensive cognitive benefit result in the short run, but other effects such as cytoprotection or disease stabilization may be manifest with long-term stimulation (based on the data suggesting that nicotinic stimulation may be cytoprotective against β -amyloid toxicity) (Kihara et al 1997; Zamani et al 1997).

Conclusions

A decade and a half of remarkable basic scientific work has resulted in substantial progress in understanding the molecular structure, neuroanatomy, distribution, and physiology of CNS nicotinic receptors (Paterson and Nordberg 2000). Understanding of their functional role in human physiology, cognitive and behavioral psychology, and human pathologies has, however, lagged behind, due in large measure to technical difficulties in the study of these functions in humans and the lack of ideal pharmacologic, imaging, and cognitive tools for the analysis and assessment of their properties. This latter situation is now beginning to change as the ability to image nicotinic receptor subtypes in the human brain becomes possible with more selective ligands (Sihver et al 1999), increasingly sophisticated electrophysiologic techniques being applied (Adler et al 1992, 1993), more selective cognitive paradigms developed, and the availability of more subtype-selective nicotinic agonists (Lloyd and Williams 2000) and allosteric modulators for human use that will allow a more precise definition of nicotinic receptor contribution to attention, learning, memory, and behavior.

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References

- Adler LE, Hoffer LD, Wiser A, Freedman R (1993): Normalization of auditory physiology by cigarette smoking in schizophrenic patients. *Am J Psychiatry* 150:1856-1861.
- Adler LE, Hoffer LJ, Griffith J, et al (1992): Normalization by nicotine of deficient auditory sensory gating in the relatives of schizophrenics. *Biol Psychiatry* 32:607-616.
- Aisen PS, Davis KL, Berg JD, et al (2000) A randomized

- controlled trial of prednisone in Alzheimer's disease. *Neurology* 54:588-593.
- Arendash GW, Sengstock GJ, Sanberg PR, Kem WR (1995): Improved learning and memory in aged rats with chronic administration of the nicotinic receptor agonist GTS-21. *Brain Res* 674:252-259.
- Arneric SP, Anderson D, Bannon A, et al (1995): Preclinical pharmacology of ABT-418: A prototypical cholinergic channel activator for the potential treatment of Alzheimer's disease. *CNS Drugs Rev* 1:1-26.
- Aubert I, Araujo DM, Cécyre D, et al (1992): Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. *J Neurochem* 58:529-541.
- Baddeley A (1988): Cognitive psychology and human memory. *Trends Neurosci* 11:176-181.
- Bartus R, Dean R, Beer B, et al (1982): The cholinergic hypothesis of geriatric memory dysfunction. *Science* 217:408-417.
- Bencherif M (1999, May): Targacept compounds potentiate sub-maximal doses of tacrine and aricept. Presented at IBC 2nd International Symposium on Nicotinic Acetylcholine Receptors: Advances in Molecular Pharmacology and Drug Development, Annapolis, Maryland.
- Brioni JD, O'Neill AB, Kim DJB, et al (1994): Anxiolytic-like effects of the novel cholinergic channel activator ABT 418. *J Pharmacol Exp Ther* 271:353-361.
- Buccafusco JJ, Jackson WJ, Terry Jr AV, et al (1995): Improvement in performance of a delayed matching-to-sample task by monkeys following ABT-418: A novel cholinergic channel activator for memory enhancement. *Psychopharmacology* 120:256-266.
- Drachman D, Leavitt J (1974): Human memory and the cholinergic system. *Arch Neurol* 30:113-121.
- Driscoll P (1976): Nicotine-like behavioral effect after small dose of mecamylamine in Roman high-avoidance rats. *Psychopharmacologia* 46:119-121.
- Flynn D, Mash D (1986): Characterization of 1-[³H]nicotine binding in human cerebral cortex: Comparison between Alzheimer's disease and the normal. *J Neurochem* 47:1948-1954.
- Fryer JD, Lukas RJ (1999): Noncompetitive functional inhibition at diverse, human nicotinic acetylcholine receptor subtypes by bupropion, phencyclidine, and ibogaine. *J Pharmacol Exp Ther* 288:88-92.
- Fujii S, Jia Y, Yang A, Sumikawa K (2000): Nicotine reverses GABAergic inhibition of long-term potentiation induction in the hippocampal CA1 region. *Brain Res* 863:259-265.
- Heishman SJ, Taylor RC, Henningfield JE (1994): Nicotine and smoking: A review of effects on human performance. *Exp Clin Psychopharmacol* 2:345-395.
- Higgins ST, Woodward BM, Henningfield JE (1989): Effects of atropine on repeated acquisition and performance of response sequences in humans. *J Exp Anal Behav* 51:5-15.
- James JR, Nordberg A (1995): Genetic and environmental aspects of the role of nicotinic receptors in neurodegenerative disorders: Emphasis on Alzheimer's disease and Parkinson's disease. *Behav Genet* 25:149-159.
- Jones GMM, Sahakian BJ, Levy R, et al (1992): Effects of acute subcutaneous nicotine on attention, information processing and short term memory in Alzheimer's disease. *Psychopharmacology* 108:485-494.
- Kelton MC, Kahn HJ, Conrath CL, Newhouse PA (2000): The effects of nicotine on Parkinson's disease. *Brain Cogn* 43:274-282.
- Kihara T, Shimohama S, Sawada H, et al (1997): Nicotinic receptors stimulation protects neurons against beta-amyloid toxicity. *Ann Neurol* 42:159-163.
- Lipiello PM, Bencherif M, Gray JA, et al (1996): RJR-2403: A nicotinic agonist with CNS selectivity II: In vivo characterization. *J Pharmacol Exp Ther* 279:1422-1429.
- Little JT, Johnson DN, Minichiello M, et al (1998): Combined nicotinic and muscarinic blockade in elderly normal volunteers: Cognitive, behavioral, and physiological responses. *Neuropsychopharmacology* 19:60-69.
- Lloyd GK, Menzaghi F, Bontempi B, et al (1998): The potential of subtype-selective neuronal nicotinic acetylcholine receptor agonists as therapeutic agents. *Life Sci* 62:1601-1606.
- Lloyd GK, Williams M (2000): Neuronal nicotinic receptors as novel drug targets. *JPET* 292:461-467.
- Maelicke A, Albuquerque EX (2000): Allosteric modulation of nicotinic acetylcholine receptors as a treatment strategy for Alzheimer's disease. *Eur J Pharmacol* 393:165-170.
- Mancuso G, Andres P, Ansseau M, Tirelli E (1999a): Effects of nicotine administered via a transdermal delivery system on vigilance: A repeated measure study. *Psychopharmacology (Berl)* 142:18-23.
- Mancuso G, Warburton DM, Melan M, et al (1999b): Selective effects of nicotine on attentional processes. *Psychopharmacology (Berl)* 146:199-204.
- McClure DE (1999, May): The potential therapeutic usefulness of subtype selective neuronal nicotinic acetylcholine receptor agonists for the motor, cognitive, and disease progression components of Parkinson's disease. Presented at IBC 2nd International Symposium on Nicotinic Acetylcholine Receptors: Advances in Molecular Pharmacology and Drug Development, Annapolis, Maryland.
- Mulnard RA, Cotman CW, Kawas C, et al (2000): Estrogen replacement therapy for treatment of mild to moderate Alzheimer's disease: A randomized controlled trial. *JAMA* 283:1007-1015.
- Newhouse PA, Kelton M (2000): Clinical aspects of nicotinic agents: Therapeutic applications in central nervous system disorders. In: Clementi F, Gotti C, Fornasari D, editors. *Handbook of Experimental Pharmacology: Neuronal Nicotinic Receptors*. Heidelberg, Germany: Springer, 779-812.
- Newhouse PA, Potter A, Corwin J, et al (1992): Acute nicotinic blockade produces cognitive impairment in normal humans. *Psychopharmacology* 108:480-484.
- Newhouse PA, Potter A, Corwin J, et al (1994): Age-related effects of the nicotinic antagonist mecamylamine on cognition and behavior. *Neuropsychopharmacology* 10:93-107.
- Newhouse PA, Potter A, Corwin J, et al (1996): Effects of nicotinic cholinergic agents on cognitive functioning in Alzheimer's and Parkinson's disease. *Drug Dev Res* 38:278-289.
- Newhouse PA, Potter A, Lenox RH, et al (1993): Effects of nicotinic agents on human cognition: Possible therapeutic applications in Alzheimer's and Parkinson's diseases. *Med Chem Res* 2:628-642.

- Newhouse PA, Sunderland T, Narang PK, et al (1990): Neuroendocrine, physiologic, and behavioral responses following intravenous nicotine in nonsmoking healthy volunteers and in patients with Alzheimer's disease. *Psychoneuroendocrinology* 15:471-484.
- Newhouse PA, Sunderland T, Tariot PN, et al (1988): Intravenous nicotine in Alzheimer's disease: A pilot study. *Psychopharmacology* 95:171-175.
- Nordberg A (1993): Effect of long-term treatment with tacrine (THA) in Alzheimer's disease as visualized with PET. *Acta Neurol Scand Suppl* 149:62-65.
- Nordberg A, Lundqvist H, Hartvig P, et al (1995): Kinetic analysis of regional (S) (-) ¹¹C-nicotine binding in normal and Alzheimer brains—in vivo assessment using positron emission tomography. *Alzheimer Dis Assoc Disord* 9:21-27.
- Papke RL, Thinschmidt JS, Moulton BA, et al (1997): Activation and inhibition of rat neuronal nicotinic receptors by ABT-418. *Br J Pharmacol* 120:429-438.
- Paterson D, Nordberg A (2000): Neuronal nicotinic receptors in the human brain. *Prog Neurobiol* 61:75-111.
- Perkins KA (1999): Baseline-dependency of nicotine effects: A review. *Behav Pharmacol* 10:597-615.
- Perry EK, Morris CM, Court JA, et al (1995): Alteration in nicotinic binding sites in Parkinson's disease, Lewy body dementia, and Alzheimer's disease: Possible index of early neuropathology. *Neuroscience* 64:385-395.
- Potter A, Corwin J, Lang J, et al (1999): Acute effects of the selective cholinergic channel activator (nicotinic agonist) ABT-418 in Alzheimer's disease. *Psychopharmacology* 142:334-342.
- Predergast MA, Jackson WJ, Terry AV, et al (1998): Central nicotinic receptor agonists ABT-418, ABT-089, and (-)nicotine reduce distractibility in adult monkeys. *Psychopharmacology* 136:50-58.
- Robbins TW, Semple J, Kumar R, et al (1997): Effects of scopolamine on delayed-matching-to-sample and paired associates tests of visual memory and learning in human subjects: Comparison with diazepam and implications for dementia. *Psychopharmacology* 134:95-106.
- Rogers J (2000, April): Therapeutic approaches to Alzheimer's disease inflammation. Presented at the Sixth International Stockholm/Springfield Symposium on Advances in Alzheimer Therapy, Stockholm.
- Sahakian BJ, Coull JT (1994): Nicotine and THA: Evidence for improved attention in patients with dementia of the Alzheimer type. *Drug Dev Res* 31:80-88.
- Sanberg PR, Shytle RD, Silver AA (1998): Treatment of Tourette's syndrome with mecamylamine. *Lancet* 352(9129):705-706.
- Sihver W, Fasth KJ, Ögren M, et al (1999): In vivo positron emission tomography studies on the novel nicotinic receptor agonist [¹¹C] MP compared with [¹¹C] ABT and (S) (-)[¹¹C] nicotine in rhesus monkeys. *Nucl Med Biol* 26:633-640.
- Snaedal J, Johannesson T, Jonsson JE, et al (1996): The effects of nicotine in dermal plaster on cognitive functions in patients with Alzheimer's disease. *Dementia* 7:47-52.
- Snodgrass J, Corwin J (1988): Pragmatics of recognition memory: Application to dementia and amnesia. *J Exp Psychol Gen* 117:34-50.
- Sunderland T, Tariot PN, Newhouse PA (1989): Differential responsivity of mood, behavior, and cognition to cholinergic agents in elderly neuropsychiatric populations. *Brain Res Rev* 12:371-389.
- Terry AV, Buccafusco JJ, Predergast MA (1999): Dose-specific improvements in memory-related performance by rats and aged monkeys administered the nicotinic-cholinergic antagonist mecamylamine. *Drug Dev Res* 47:127-136.
- Thal LJ, Forrest M, Loft H, Mengel H (2000): Lu 25-109, a muscarinic agonist, fails to improve cognition in Alzheimer's disease Lu25-109 Study Group. *Neurology* 54:421-426.
- Warburton DM, Rusted JM (1993): Cholinergic control of cognitive resources. *Neuropsychobiology* 28:43-46.
- Warman U, Nordberg A (1995): Epibatidine and ABT 418 reveal selective losses of alpha 4 beta 2 nicotinic receptors in Alzheimer brains. *Neuroreport* 6:2419-2423.
- Weinstock M (1999): Selectivity of cholinesterase inhibition clinical implications for the treatment of Alzheimer's disease. *CNS Drugs* 4:307-323.
- White HK, Levin ED (1999): Four-week nicotine skin patch treatment effects on cognitive performance in Alzheimer's disease. *Psychopharmacology* 143:158-165.
- Whitehouse P, Martino A, Antuono P, et al (1986): Nicotinic acetylcholine binding sites in Alzheimer's disease. *Brain Res* 371:146-151.
- Whitehouse PJ, Hedreen JC, White CL, et al (1983): Basal forebrain neurons in dementia of Parkinson's disease. *Ann Neurol* 13:243-248.
- Whitehouse PJ, Martino AM, Wagster MV, et al (1988): Reductions in [³H] nicotinic acetylcholine binding in Alzheimer's disease and Parkinson's disease: An autoradiographic study. *Neurology* 38:720-723.
- Wilens T, Biederman J, Spencer T, et al (1998): A controlled trial of ABT-418 in attention deficit hyperactivity disorder in adults. *Am J Psychiatry* 156:1931-1937.
- Wilson AL, Langley LK, Monley J, et al (1995): Nicotine patches in Alzheimer's disease: Pilot study on learning, memory, and safety. *Pharmacol Biochem Behav* 51:509-514.
- Xiang Z, Huguenard J, Prince D (1998): Cholinergic switching within neocortical inhibitory networks. *Science* 281:985-988.
- Zamani MR, Allen YS, Owen GP, Gray JA (1997): Nicotine modulates the neurotoxic effect of beta-amyloid protein (25-35) in hippocampal cultures. *Neuroreport* 8:513-517.

ORIGINAL INVESTIGATION

Alexandra Potter · June Corwin · Jason Lang
Melissa Piasecki · Robert Lenox · Paul A. Newhouse

Acute effects of the selective cholinergic channel activator (nicotinic agonist) ABT-418 in Alzheimer's disease

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Abstract To explore further the potential for cognitive enhancement utilizing nicotinic stimulation in Alzheimer's disease (AD), six otherwise healthy subjects with moderate AD received placebo and three doses (6, 12, and 23 mg) of the novel selective cholinergic channel activator (ChCA), (nicotinic agonist) ABT-418 over 6 h in a double-blind, within-subjects, repeated-measures design. Subjects showed significant improvements in total recall and a decline in recall failure on a verbal learning task. Qualitatively similar improvements were seen in non-verbal learning tasks such as spatial learning and memory, and repeated acquisition. No significant behavioral, vital sign, or physical side effects were seen. These results confirm that stimulating central nicotinic receptors has acute cognitive benefit in AD patients. These findings suggest that selective ChCAs have a potential therapeutic role in dementing disorders, and that further studies with this or similar agents in AD and/or Parkinson's disease are warranted.

Key words Alzheimer's disease · ABT-418 · Novel nicotinic agonist · Learning · Memory

Introduction

Alzheimer's disease (AD) is characterized by numerous neurochemical deficits associated with cellular derangement (Palmer and Gershon 1990; Blennow et al. 1996). Historically, therapeutic research has highlighted the role of the muscarinic cholinergic system in the modulation of cognitive and behavioral symptoms of this disease (Nordberg 1994). More recently, attention has focused on elucidating the role of the nicotinic cholinergic system in cognitive functioning and in specific cognitive disorders such as AD (Newhouse et al. 1997).

Patients with AD show a marked reduction in cortical nicotinic cholinergic receptor binding compared to age-matched controls (Flynn and Mash 1986; Whitehouse et al. 1986; Perry et al. 1987; Giacobini 1990). Investigators have studied the functional role that nicotinic receptors may play in both normal and diseased human cognition by evaluating the effects of nicotinic agonists and antagonists on cognitive functioning in AD patients, as well as young and elderly normals (Newhouse et al. 1988, 1990, 1992, 1993, 1994; Jones et al. 1992; Nordberg 1994; Wilson et al. 1995). The results of these investigations indicate that nicotinic receptor system functioning is important in attention (Jones et al. 1992), verbal learning (Newhouse et al. 1988, 1992; Jones et al. 1992), spatial memory (Decker et al. 1992), and psycho-motor speed (Newhouse et al. 1994). Early animal studies show that nicotine facilitates task acquisition and memory consolidation (Nelson and Goldstein 1972; Nordberg and Bergh 1989), and improves performance on delayed match-to-sample tasks in monkeys (Elrod et al. 1988). While the cognitive domains that are associated with loss of nicotinic receptor binding are becoming better understood, research into therapeutic nicotinic augmentation is increasingly an area of active investigation. Recent advances in understanding of the structure, function and distribution of CNS nicotinic

A. Potter · J. Lang · M. Piasecki · P.A. Newhouse (✉)
Clinical Neuroscience Research Unit, Department of Psychiatry,
University of Vermont College of Medicine,
1 South Prospect Street, Burlington, VT 05401, USA
e-mail: pnewhou@zoo.uvm.edu, Fax: +1-802-656-7889

J. Corwin
Department of Psychiatry,
New York Veteran's Affairs Medical Center,
New York University School of Medicine, New York,
New York, USA

R. Lenox
Department of Psychiatry, University of Pennsylvania College
of Medicine, Philadelphia, Pennsylvania, USA

receptors and the differences between molecular receptor subtypes (Gotti et al. 1997) have provided an additional impetus for intensifying these research efforts.

While there is theoretical support for the therapeutic use of nicotinic agents for the cognitive and behavioral symptoms of AD, the administration of nicotine is problematic due to its narrow therapeutic index, gastrointestinal and autonomic toxicity, and a steep dose-response curve for cognitive effects (Newhouse et al. 1997). With an increased understanding of the molecular and structural biology of the nicotinic receptor, attention has recently focused on the development of compounds that may be more selective and have a greater therapeutic index (Arneric et al. 1995). ABT-418 is a novel nicotine derivative with highly selective binding to central nicotinic (cholinergic channel) receptors and no significant activity at dopamine, serotonin, muscarinic, GABA, or other G-protein linked receptors or ligand-gated ion channels thus far examined. It shows selectivity for the [^3H]cytisine-labeled nicotine binding site but is minimally active at the neuromuscular junction or α -bungarotoxin-sensitive nicotinic receptor in vitro (Arneric et al. 1994). ABT-418 produces some in-vitro effects that are similar to those produced by nicotine, but with findings suggestive of greater selectivity for the $\alpha 4\beta 2$ nicotinic receptor subtype and less selectivity for the $\alpha 3$ subunit (Arneric et al. 1994). In animal studies, ABT-418 produces effects on behavior, locomotor activity and learning that are similar to nicotine, but with a considerably larger therapeutic index and generally more robust effects on learning and memory, as well as anxiolytic effects (Decker et al. 1994). Initial studies in normal humans suggest that the agent is well tolerated (Sebree et al. 1993). Overall, the profile suggests that ABT-418 may be a promising selective nicotinic agonist for clinical and research investigations (Arneric et al. 1994).

Thus, we began a study of the acute effects of ABT-418 in patients with early AD by examining the specific domains of cognitive and behavioral functioning that appear potentially modifiable through administration of a selective agonist at cholinergic channel (nicotinic) receptors.

Materials and methods

Subjects

Six non-smoking patients (four females and two males) with a mean age of 72.7 (± 10.7) participated in this study. All subjects met criteria for NINDS-ADRDA diagnosed probable Alzheimer's disease (McKhann et al. 1984). Subjects had a mean global deterioration score (Reisberg et al. 1982) of 3.2, indicating mild dementia. Mean Mini-Mental State score (Folstein et al. 1975) was 21.4 (± 3.0). Subjects were determined by physical examination and laboratory tests to be free of other unstable medical or active psychiatric illnesses. This study was approved by the University of Vermont Institutional Review Board and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Informed

consent was obtained from all patients and appropriate family members.

Cognitive test battery

The cognitive battery was designed to assess verbal and non-verbal memory, sustained attention, and reaction time. The tests selected have been used in previous drug studies and have been shown to be sensitive to nicotinic stimulation and/or blockade in young and elderly healthy volunteers as well as patients with AD and Parkinson's disease (PD) (Newhouse et al. 1992, 1994). Pre-testing training was done with every subject prior to each experiment so as to ensure stable asymptotic performance and minimal practice effects. All testing was done double blind to drug dosage.

Verbal learning and memory

Selective reminding task

A seven-item selective reminding task was used to test learning and recall. This standard test has been widely used in studies of cognitive impairment and offers measures of storage into and retrieval from both short-term and long-term memory (Buschke and Fuld 1974). Administration of this test consists of the technician reading seven words to the subject and the subject immediately recalling as many words as possible. The technician then presents an adjusted list containing only the items that were omitted by the subject from the original list at the last trial. The subject then tries to recall the entire list (all seven words). This is repeated for a total of eight trials, or until the subject recalls all items on the list. Measures obtained include total recall, recall consistency (a measure of how long words are held in memory unreinforced), and recall failure, defined as failing to recall a word after two successive reinforcements.

Recognition memory

The High-Low Imagery Test (Corwin et al. 1987) was administered to test recognition memory. In this test subjects are presented with 14 target words, seven each of high imagery ("cat") and low imagery ("idea"). During the recognition test subjects are presented with 28 words, the original 14 targets and 14 distractors (seven high and seven low imagery), and asked to indicate which words are old and new. In this study, the subjects were presented with two immediate recognition trials (in which they saw the list to learn and immediately after completed the recognition test described above) and then a delayed recognition trial approximately 15 min later in which they completed the recognition test without an additional presentation of the target list. This task permits independent assessment of two aspects of cognition: the course of acquisition and forgetting of the stimulus material (discrimination) and the performance behavior of the subject when he/she is uncertain (response bias).

Non-verbal learning and memory

Repeated acquisition test

This procedure (RAT) allows the simultaneous measurement of a subject's ability to retrieve previously acquired non-verbal information as well as his ability to learn new information (Thompson 1973; Higgins et al. 1989). In this procedure, a subject learns a sequence of button pushes (1-10) on a three-button panel, with reinforcement provided by a computer (training phase). During the drug study, the subject then enters a second phase (learning and

performance phase). In the performance condition, the correct response sequence is always the same (the originally learned chain). In the learning condition, a new response sequence must be learned each time the task is done. During testing, the subject alternates between the performance and learning conditions. This allows the assessment of state changes on both new learning and recall of old learning almost simultaneously. Measures obtained include number of errors made over 20 trials, and quarterlife, defined as the trial by which one quarter of the total errors are made.

Spatial memory task

This task, developed by one of the authors (J.C.) is adapted from the animal work of Robbins and associates (1990). Administration involves five large squares appearing in a line across a computer screen. After a warning tone, one square turns black for either 0.5 or 1 s. A gray mask then descends down the screen and stays visible for 0.1, 2, 5, 7, or 9 s. After the mask ascends, the display of squares reappears with a warning tone and the statement "CLICK ON THE SQUARE THAT WAS BLACK". The subject then clicks with the mouse on the appropriate square which turns black briefly. If a response is not made within 5 s the next trial starts. Data obtained from this task include hits, misses, and reaction time.

Psychomotor ability and attention

A 36 hit "A-X" Continuous Performance Task was used. The subject is required to monitor the screen while a series of letters is displayed one at a time on the screen. The subject is instructed to press a mouse button when they see an "A" followed by an "X". Stimuli were presented on the screen for a minimum of 0.5 s until a response (3-s maximum) with a 0.1-s interstimulus interval. The total task duration was 10 min. This task measures attentional capability, short-term memory, and psychomotor speed. Hits, false alarms and reaction time were examined.

Behavioral measures

For observer ratings, the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham 1962) was used to measure psychopathologic behaviors. This scale appears to be sensitive to the behavioral changes induced by cholinergic drugs in both normals and AD patients (Sunderland et al. 1989). A battery of visual analog scales (VAS) consisting of a series of items such as "drowsiness" or "psychomotor agitation", were scored on 100 mm lines. For the subject, a visual analog battery (VAB) of self-report items and a physical symptom checklist were used.

Drug

ABT-418 was administered through the skin via a transdermal device for 6 h. All study drug was administered double blind and doses were determined using a random order procedure. Each subject received a dose of placebo or approximately 6, 12, or 23 mg ABT-418 on each of the 4 study days.

Procedures

There were 4 dosing-days, each separated by at least 48 h. The dosing sequence was determined by a random order procedure. Following an overnight fast, the study began at 0700 hours with baseline cognitive testing and behavioral evaluation. Drug was

administered continuously for the next 6 h with cognitive testing completed at +2 and +6 h of drug administration, and again 2 h after drug administration ceased. Behavioral ratings were completed at pre-drug baseline, 2 h after administration, and 2 h after drug was withdrawn. Vital signs were obtained at 30-min intervals and included blood pressure, tympanic temperature, and pulse rate. Blood samples were drawn for cortisol, prolactin and plasma drug levels 1 h prior to the start of drug administration, hourly through 6 h, and 2 h after drug administration stopped.

Data analysis

Data were analyzed by completely repeated measures ANOVA with dose (placebo, 6, 12, and 23 mg) and time as levels of repeated factors. Significant dose-related effects were inferred by significant dose by time interaction terms in the ANOVA. Modified *F* tests were used to adjust for correlated repeated measures (Vitaliano 1982). For the Buschke Selective Reminding Task, the components (total recall and consistency) were analyzed with trial as an additional within-factor. Data is reported for the first 5 trials on this task as trials 6-8 showed no additional significant changes from trial 5. For the Hi-Low Imagery test, dependent measures included estimates of discrimination and response bias calculated using the Two-High Threshold theory (Snodgrass and Corwin 1988), per imagery condition and trial. Planned post-hoc comparisons were done utilizing Fisher's PLSD test. Where pre-drug scores showed significant between day variability, change scores between pre-drug and post-drug were used in the ANOVA.

Results

All results presented consist of data from the 6-hour testing session unless otherwise indicated.

Verbal learning and retrieval

On the Selective Reminding Task there was a significant ($F_{9,60} = 1.97$, $P = 0.02$) dose by time interaction on the total number of words correctly recalled through five trials. Recall was significantly better after the 23 mg dose of ABT-418 (20.17 ± 1.5) than after placebo (16.6 ± 2.03). When scores were adjusted for baseline performance, the values showed a linear dose-response relationship with change scores ranging from negative to positive with increasing dose, (Table 1, Fig. 1).

There was also a significant ($F_{9,60} = 2.98$, $P = 0.005$) dose by time interaction on recall failures on this task (Table 1, Fig. 2). A recall failure is indicative of the inability to retain a word in and/or recall it from working memory after at least two successive reinforcements. This indicates either a failure of acquisition (through encoding failure or inattention) or a capacity problem with working memory. Post-hoc testing showed that the 23 mg dose produced significantly ($P = 0.0005$) fewer recall failures (1.3 ± 0.9) than placebo (5.0 ± 2.0). Analysis of change scores showed a dose-related decline (Fig. 2).

A non-significant linear dose-related increase in recall consistency (a measure of how long words are

Table 1. Selected cognitive findings. SRT Selective Reminding Task, SMT Spatial Memory Task, RAT Repeated Acquisition Task, CPT Continuous Performance Task. 2 h post = 2 h after drug administration was stopped

	SRT recall				SRT recall-failure				SMT hits				RAT errors ^b				CPT mean rt			
	Placebo	6 mg	12 mg	23 mg	Placebo	6 mg	12 mg	23 mg	Placebo	6 mg	12 mg	23 mg	Placebo	6 mg	12 mg	23 mg	Placebo	6 mg	12 mg	23 mg
Baseline	20.3	19.3	18.2	17.3	0.5	1.3	2.8	4.0	47.50	48.33	46.00	46.83	8.83	13.50	11.17	13.00	0.633	0.662	0.731	0.696
+2 h	18.7	19.0	19.0	18.5	1.7	2.7	2.0	2.7	46.67	43.33	46.00	49.17	10.33	10.33	10.00	9.00	0.645	0.702	0.618	0.634
+6 h	16.6	18.3	18.6	20.1	5.0	3.3	2.5	1.3	41.83	44.17	46.33	48.17	11.17	9.00	12.40	10.67	0.615	0.633	0.647	0.621
2 h post	17.0	19.2	20.0	16.0	2.3	1.2	1.2	4.8	44.17	49.17	48.83	47.67	11.00	8.20	6.83	7.67	0.620	0.698	0.641	0.650
46 h ^a	-3.8	-1.0	+0.5	+2.8	+4.5	+2.0	-0.3	-2.7	-5.67	-4.17	+0.33	+1.33	+2.34	-4.50	+1.23	-2.33	-0.018	-0.027	-0.084	-0.075

^a46 h change from pre-drug baseline to 6-h testing

^bThese data exclude one subject (see text)

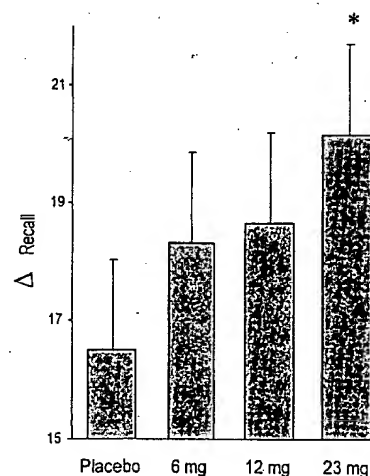


Fig. 1 Selective reminding task - total words recalled (over first five trials). * $P < 0.05$ different from placebo

held in memory when they are not being reinforced) was also seen.

Significant effects of drug administration on this task began to disappear 2 h after discontinuation of drug administration. This effect was more marked after higher doses of ABT-418 with a greater regression seen in scores toward placebo values. For example, the difference between the placebo and 23 mg change scores on the Recall Failure measure decreased from 2.7 at 6 h to 1.0 at 2 h after drug administration had ceased.

The High-Low Imagery task showed a trend ($P = 0.08$) toward a dose related increase in high imagery hits at the 6-h time point with hits of 4.5 on placebo, 6.2 at 6 mg, 5.75 at 12 mg and 5.5 at 23 mg. Examination of the amount of information forgotten during the delay period (score at trial 2 - score at delay) indicated that there was more forgetting following placebo administration than following the three active doses of ABT-418. Administration of ABT-418 also produced a trend ($P = 0.08$) toward an effect on response bias on this task. The response bias for low imagery words became more conservative after all active doses of ABT-418, and more liberal after administration of placebo during the delay phase (Fig. 3).

Non-verbal learning and memory

The Spatial Memory task demonstrated a dose-related increase in correct identifications with hits increasing with ascending doses of ABT-418 (Table 1). This trend continued 2 h after the cessation of drug administration. Change-from-baseline scores showed that the decline in performance over time seen after placebo did not occur with the middle and high doses of ABT-418. False alarm scores did not change in any meaningful way.

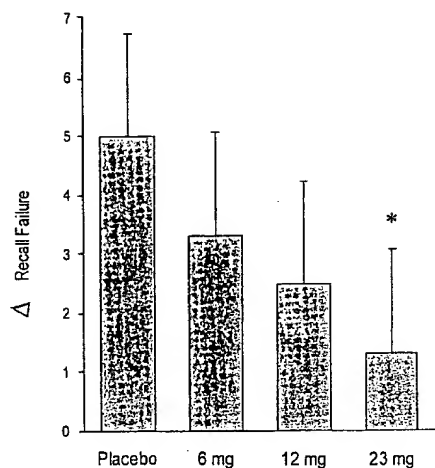


Fig. 2 Selective reminding task - recall failures (over first five trials). * $P < 0.05$ difference from placebo

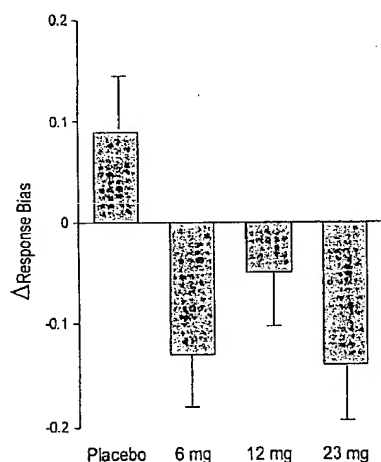


Fig. 3 High-low imagery task - response bias - shift during delay period. (Negative scores represent conservative shift of response bias)

On the Repeated Acquisition Task all active drug doses decreased total errors in both the learning and performance condition. In addition, on inspection of the data, one subject was found to have performed at ceiling on the new learning portion of this task at all time points. When her data were excluded from the statistical analysis all active doses prevented the decline in performance seen on placebo ($F_{3,31} = 3.44$, $P = 0.05$) (Table 1).

Performance change scores showed a trend ($P < 0.08$) for a similar effect with all active drug change scores below 0 versus a positive score on placebo. Quarter-life calculations showed slightly lower change scores at the highest dose (23 mg).

Psychomotor speed

Analysis of change scores in reaction time on the Continuous Performance Task at 6 h showed a trend ($P < 0.10$) for a decrease in reaction time from baseline with ascending doses of drug (Table 1) up to the middle dose.

There was no difference in reaction times for hits or false alarms. A trend ($P = 0.09$) towards a dose-related decrease in reaction time (from 2.35 to 2.15 s) was also seen on the spatial memory task.

Behavioral measures

The subjective visual analog scale demonstrated a trend ($P = 0.12$) towards a dose-related decrease in feelings of anxiety at the 2-h time point. Change scores showed a reduction of self-rated anxiety at all active doses of ABT-418 (Table 2). Supporting this finding was a reduction of self-rated fear seen after the high (23 mg) dose of ABT-418 (Table 2). This dose reversed the increase in fear that was evident after administration of placebo and the lower doses of ABT-418 (Table 2). No significant changes were seen on blinded observer ratings of mood, anxiety, or behavior.

Physiological measures

There were no significant dose-related changes seen in any of the vital sign measures. These included blood pressure, tympanic temperature and pulse. Monitoring of heart rhythm via on-line cardiac telemetry revealed no effects of ABT-418 on cardiac rhythm. Further, no significant adverse events occurred at any dose. Specifically, no effects were seen on gastrointestinal functioning, with no nausea, vomiting, or defecation. Patients were not reliable in distinguishing which dose of drug they received.

Table 2 Behavioral findings.
SVA Subjective visual analog

	SVA anxiety				SVA fear			
	Placebo	6 mg	12 mg	23 mg	Placebo	6 mg	12 mg	23 mg
2 h	27.33	23.33	25.17	15.33	23.83	27.67	25.66	8.5
Change	+2.5	-13.67	-3.17	-6.67	+8.5	+9.5	+9.34	-4.67

Table 3 ABT-418 plasma levels (ng/ml) by dose at selected time points

	Placebo	6 mg	12 mg	23 mg
Baseline	0.0	0.0	0.0	0.80
2 h	0.0	4.01	6.04	13.76
6 h	0.0	8.26	10.68	28.54
2 h post	0.0	4.73	7.75	19.80

Hormonal measures

Samples were drawn hourly for cortisol and prolactin. No significant effect of ABT-418 was seen on either hormone. There was a slight elevation of prolactin after the 23 mg dose at +1 and +2 h, but this was not sustained. Cortisol levels were maximal at baseline for all doses and tended to decline over the day on all doses.

Drug levels

Plasma levels of ABT-418 were obtained hourly through 8 h. Peak plasma levels were observed at 6 h and were non-linear (Table 3). Levels taken at 8 h showed that plasma levels had declined an average of 34%, 2 h after cessation of drug administration.

Discussion

The most robust effects seen in response to acute administration of ABT-418 in AD patients were improvements in the selective reminding task (a measure of verbal learning and recall). Specifically, we found that ABT-418 administration lead to an increase in total number of words recalled, and a decline in recall failures. Improvements on these measures suggest enhancement of attention and/or acquisition mechanisms and/or improvements in the so-called "phonological loop" of working memory (Baddeley 1988). Consistent with improvements in verbal learning and immediate recall were the results of the spatial memory, recognition memory, and RAT tasks. Although the effects seen on these tasks were not as robust as the SRT effects, all three tasks showed dose-related effects that trended in the same direction as the SRT. These results provide further support for ABT-418 increasing attentional function and/or working memory, perhaps through actions on forebrain dopamine systems.

Interestingly, greater improvements were seen on accuracy in the more effort-demanding SRT as compared to the verbal recognition memory task. Recognition memory tasks are not as effortful and do not require an elaborate retrieval strategy because the retrieval cue is presented. The lack of improvement of

the recognition memory task argues against mere attentional or arousal effects of ABT-418. The differences in effects on the two tasks suggest that ABT-418 may have effects at the encoding stage of memory formation as well as the retrieval stage. Consistent with these findings, our prior research has shown that the nicotinic antagonist mecamylamine (Newhouse et al. 1993, 1994, 1995) produces impairment on the SRT in precisely the opposite direction, i.e. an increase in recall failure, decrease in recall consistency, and a decrease in total correct recall. Additionally, in a prior study using intravenous nicotine in patients with AD (Newhouse et al. 1988), we showed that nicotine selectively decreased recall intrusion errors in a verbal free recall task, suggesting an effect on working memory and/or retrieval mechanisms.

On the recognition memory task there was a strong trend for the highest dose of ABT-418 to change the response bias measure (Br) in a more conservative direction. Alzheimer's disease patients typically show an inappropriately liberal decision bias (Snodgrass and Corwin 1988) on binary decision tasks, reflecting impaired decision-making or response selection mechanisms. Previously we have shown that the nicotinic antagonist mecamylamine produces a liberal shift in response bias measures on this task in elderly normals (Newhouse et al. 1994) and increases false alarms in AD patients (Newhouse et al. 1993). The effect of ABT-418 on response bias (correcting for an overly liberal bias) in patients with AD may reflect an improved response or recall selection mechanism that is modulated through nicotinic receptors. Such a mechanism may be mediated through catecholamine systems, as amphetamine has been shown to normalize abnormal response bias in states of cognitive impairment such as following prolonged sleep deprivation (Newhouse et al. 1989).

In our studies with the nicotinic antagonist mecamylamine, we showed that non-verbal learning and memory was impaired using the RAT task (Newhouse et al. 1993, 1994, 1995). This impairment was dose- and age-related, with an increasing impairment with dose, age and/or disease. This impairment was manifested largely through an increase in the errors on this task, which included slower learning of the chain, and difficulty holding the learned sequence in working memory, i.e., errors would continue to occur even after the chain was initially acquired. ABT-418 decreased errors at all doses on this task, but the effect was not as robust as on the verbal learning tasks. This task requires that the subject pay strict attention to reinforcement (or lack of it) from the screen and there is less assistance from a technician than the SRT, which is administered orally. It may be that highly structured tasks with technician involvement (potentially decreasing attentional requirements) respond better to nicotinic stimulation than relatively less structured ones or that verbal learning is more likely to show

enhancement with nicotinic stimulation in humans than non-verbal learning.

Similar to previous studies with nicotine, there was a strong trend for a dose-related improvement in the speed of responses on the CPT task. Improvements in RT and/or speed are one of the most reliable effects of nicotinic stimulation in humans (Wesnes and Warburton 1983). It is intriguing that the effects on RT were proportionately less than effects seen on learning. It may be that the learning effects of ABT-418 are more prominent acutely than speed effects as compared to nicotine, because of its more selective receptor profile or that our tasks were not sensitive enough to detect small changes in speed.

Animal studies with ABT-418 have shown that this compound produces non-benzodiazepine-like anxiolytic effects (Decker et al. 1994). Consistent with that profile, declines in self-rated anxiety and fear were seen after the highest dose of ABT-418. However, it should be noted that the patients and the experimental conditions in this study were chosen to minimize anxiety. Nonetheless, anxiety and agitation are often associated with AD and are significant complications of this disease. A cognitive enhancer that is also anxiolytic would have significant clinical advantages.

No significant adverse reactions were seen, particularly no gastrointestinal effects. In contrast, intravenous nicotine can produce nausea and/or vomiting at doses that are very close to those necessary to see cognitive enhancement. Again in contrast to nicotine, no significant effects were seen on any vital sign measures, suggesting little ganglionic stimulation, arguing for ABT-418's receptor selectivity.

The lack of effect on cortisol and prolactin secretion is intriguing. We have shown previously (Newhouse et al. 1990) that acute intravenous administration of nicotine produces a significant rise in ACTH and cortisol, with a variable effect on prolactin in both normals and early AD patients. Therefore, the lack of response after ABT-418, especially in cortisol secretion, is unlikely to be due to pituitary insensitivity to nicotinic stimulation, but may be secondary to either the different pharmacokinetic conditions of the present experiment (slow transcutaneous administration) or more selectivity on the part of this novel nicotinic agonist. It may be that by virtue of its receptor selectivity, ABT-418 does not stimulate the pituitary at doses that produce cognitive effects, or that the conditions of the experiment were conducive to rapid tachyphylaxis or hysteresis, which has been seen with other nicotine-induced physiological responses (Porchet et al. 1988; Newhouse et al. 1992). It is also possible that lack of significant ganglionic effects may reduce peripheral feedback, which may in part be responsible for nicotine-induced pituitary hormonal secretion. The lack of adverse side-effects suggest that at least acutely, ABT-418 has a wider therapeutic index than nicotine, confirming previous animal studies (Decker et al. 1994).

Several limitations of this study suggest caution in interpretation of the results. Only six patients were studied and each patient only received a single dose at each dose level. It is unclear whether the results from this single dose study would be obtained after long-term exposure. Further, issues of long-term drug tolerability and safety were not assessed. It is possible that the results seen might disappear after longer drug exposure or that tolerance would develop to these effects. Several tasks produced results that trended in a positive direction, but the small sample and large variability of responses precluded statistical significance. A further difference from previous studies with nicotine, including our own, is worth noting. The administration time course of the nicotinic agonist ABT-418 in this study was spread over 6 h. Our prior studies of nicotine administration to AD patients (Newhouse et al. 1988, 1990, 1992, 1995) utilized a 30- to 60-min intravenous infusion route. The studies by Jones and colleagues (Jones et al. 1992; Sahakian and Coul 1994) of nicotine in AD utilized a single subcutaneous injection. Therefore, the kinetic aspects of drug administration and absorption were different between this study and prior studies with nicotine. It may be that significant effects on learning and/or memory with nicotine may require longer or more continuous administration of drug than 30-60 min. Wilson and colleagues (1995) studied nicotine administration over several weeks in AD patients and noted improvements in error rates on the Repeated Acquisition Task after 2 weeks of administration, lending support to this argument.

These data represent evidence that stimulation of nicotinic receptors can improve the acquisition and retention of verbal information (declarative memory) in humans. Previously, it has been difficult to demonstrate with nicotine itself that stimulation of nicotinic receptors produces true learning or memory improvement effects (Heishman and Henningfield 1994) and most of the cognitive effects of nicotine have been interpreted as due to attentional effects (Sahakian et al. 1994). However, as has been suggested by Warburton and Rusted (1993), nicotine's effects are most often seen in tasks that have a large attentional load and the verbal learning tasks that improved after acute administration of ABT-418 in this study required focused attention and significant cognitive effort. Further studies are required to determine if selective nicotinic stimulation by subtype-specific agonists will help determine whether nicotinic stimulation assists at maintaining or focusing attention, improves encoding, augments retrieval (from working memory), or more likely, affects a combination of processes.

These results give further support to the idea that stimulation of the nicotinic receptors of AD patients may be beneficial. It is not possible from this study to assess whether the cognitive improvements seen here will translate in to clinically manifest benefits.

Nonetheless, the possible combination of cognitive enhancement with mild anxiolytic effects is an attractive one, and longer-term studies with this agent or similar subtype-selective agonists are warranted. It is also possible that this or similar drugs may be of benefit in the treatment of PD. PD patients, especially those that become demented, appear to suffer from a similar nicotinic receptor loss as patients with AD (Aubert et al. 1992). While there are many differences between the dementia of PD and that of AD, attentional impairments may be one area of shared impairment that could be positively affected by nicotine or other cholinergic channel activators (Newhouse et al. 1997).

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References

- Arneric SP, Sullivan JP, Briggs CA, Donnelly-Roberts D, Anderson DJ, Raskiewicz JL, Hughes ML, Cadman ED, Adams P, Garvey DS, Wasicak JT, Williams M (1994) (S)-3-Methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole (ABT-418): a novel cholinergic ligand with cognition-enhancing and anxiolytic activities: I. In vitro characterization. *J Pharmacol Exp Ther* 270:310-318
- Arneric SP, Sullivan JP, Williams M (1995) Neuronal nicotinic receptors: novel targets for central nervous system therapeutics. In: Bloom F, Kupfer D (eds) *Psychopharmacology: the fourth generation of progress*. Raven Press, New York, pp 95-110
- Aubert I, Araujo DM, Cécyle D, Robitaille Y, Gauthier S, Quirion R (1992) Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. *J Neurochem* 58:529-541
- Baddeley A (1988) Cognitive psychology and human memory. *Trends Neurol Sci* 11:176-181
- Blennow K, Cowburn RF (1996) The neurochemistry of Alzheimer's disease. *Acta Neurol Scand [Suppl]* 168:77-86
- Buschke H, Fuld P (1974) Evaluating storage, retention, and retrieval in disordered memory and learning. *Neurology* 24:1019-1025
- Corwin J, Peselow E, Fieve R, Rotrosen J (1987) Memory in untreated depression: severity and task requirement effects. Presented at 26th Annual Meeting, American College of Neuropsychopharmacology, December, San Juan, PR
- Decker MW, Majchrzak MJ, Anderson DJ (1992) Effects of nicotine on spatial memory deficits in rats with septal lesions. *Brain Res* 572:281-285
- Decker MW, Brioni JD, Sullivan JP, Buckley MJ, Radek RJ, Raskiewicz JL, Kang CH, Kim DJB, Giardina WJ, Williams M, Arneric SP (1994) ABT 418: a novel cholinergic ligand with cognition enhancing and anxiolytic activities: II. In vivo characterization. *J Pharmacol Exp Ther* 270:319-328
- Elrod K, Buccafusco JJ, Jackson WJ (1988) Nicotine enhances delayed matching-to-sample performance by primates. *Life Sci* 43:277-287
- Flynn DD, Mash DC (1986) Characterization of L-[3H] nicotine binding in human cerebral cortex: comparison between Alzheimer's disease and the normal. *J Neurochem* 47:1948-1954
- Folstein MF, Folstein SE, McHugh PR (1975) "The Mini-Mental State": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12:189-198
- Giacobini E (1990) Cholinergic receptors in human brain: effects of aging and Alzheimer's disease. *J Neurosci Res* 27:548-560
- Gotti C, Fornasari D, Clementi F (1997) Human neuronal nicotinic receptors. *Prog Neurobiol* 53:199-237
- Heishman SJ, Taylor RC, Henningfield JE (1994) Nicotine and smoking: a review of effects on human performance. *Exp Clin Psychopharmacol* 2:345-395
- Higgins ST, Woodward BM, Henningfield JE (1989) Effects of atropine on the repeated acquisition and performance of response sequences in humans. *J Exp Anal Behav* 51:5-15
- Jones GMM, Sahakian BJ, Levy R, Warburton DM, Gray JA (1992) Effects of acute subcutaneous nicotine on attention, information processing and short term memory in Alzheimer's disease. *Psychopharmacology* 108:485-494
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA workshop. *Neurology* 34:939-944
- Newhouse PA (1990) Cholinergic drug studies in dementia and depression. *Adv Exp Med Biol* 282:65-76
- Newhouse PA, Sunderland T, Tariot PN, Blumhardt CL (1988) Intravenous nicotine in Alzheimer's disease: a pilot study. *Psychopharmacology* 95:171-175
- Newhouse PA, Belenky GL, Thomas M, Thorne D, Sing HC, Fertig J (1989) The effects of d-amphetamine on arousal, cognition and mood after prolonged total sleep deprivation. *Neuropsychopharmacology* 2:153-164
- Newhouse PA, Sunderland T, Narang PK, Mellow AM, Fertig JB, Lawlor BA, Murphy DL (1990) Neuroendocrine, physiologic, and behavioral responses following intravenous nicotine in non-smoking healthy volunteers and in patients with Alzheimer's disease. *Psychoneuroendocrinology* 15:471-484
- Newhouse PA, Potter A, Corwin J, Lenox R (1992) Acute nicotinic blockade produces cognitive impairment in normal humans. *Psychopharmacology* 108:480-484
- Newhouse PA, Potter A, Lenox RH (1993) Effects of nicotinic agents on human cognition: possible therapeutic applications in Alzheimer's and Parkinson's diseases. *Med Chem Res* 2:628-642
- Newhouse PA, Potter A, Corwin J, Lenox RH (1994) Age-related effects of the nicotinic antagonist mecamylamine on cognition and behavior. *Neuropsychopharmacology* 10:93-107
- Newhouse PA, Potter A, Corwin J, Lenox RH (1995) Acute cognitive effects of nicotine in Alzheimer's and Parkinson's disease. Presented at 34th Annual Meeting, American College of Neuropsychopharmacology, San Juan PR, Dec 11-15, 1995
- Newhouse PA, Potter A, Levin E (1997) Nicotinic system involvement in Alzheimer's and Parkinson's diseases: implications for therapeutics. *Drugs Aging* 11:206-228
- Nelson JM, Goldstein L (1972) Improvement of performance on an attention task with chronic nicotine treatment in rats. *Psychopharmacologia* 26:347-360
- Nordberg A (1994) Human nicotinic receptors - their role in aging and dementia. *Neurochem Int* 25:93-97
- Nordberg A, Bergh C (1985) Effect of nicotine on passive avoidance behavior and motoric activity in mice. *Acta Pharmacol Toxicol* 54:337-341
- Overall J, Gorham D (1962) The brief psychiatric rating scale. *Psychol Rep* 10:799-812
- Palmer AM, Gershon S (1990) Is the neuronal basis of Alzheimer's disease cholinergic or glutamatergic? *FASEB J* 4:2745-2752
- Perry RH, Perry EK, Smith CJ, Xuereb JH, Irving D, Whitford CA, Candy JM, Cross AJ (1987) Cortical neuropathological substrates of Alzheimer's and Parkinson's diseases. *J Neural Transm Suppl* 24:131-136
- Porchet HC, Benowitz NL, Sheiner LB (1988) Pharmacodynamic model of tolerance: application to nicotine. *J Pharmacol Exp Ther* 244:231-236
- Reisberg B, Ferris SH, DeLeon MJ, Crook T (1982) The global deterioration scale for assessment of primary degenerative dementia. *Am J Psychiatry* 139:136-139

- Robbins TW, Giardini V, Jones GH, Reading P, Sahakian B (1990) Effects of dopamine depletion from the caudate-putamen and nucleus accumbens septi on the acquisition and performance of a conditional discrimination task. *Behav Brain Res* 38:243-261
- Sahakian BJ, Coull JT (1994) Nicotine and THA: evidence for improved attention in patients with dementia of the Alzheimer type. *Drug Dev Res* 31:80-88
- Sebree T, Grebb JA, Kittle C, Kashkin KB (1993) A phase I, single, rising dose study of A-81418, a novel cholinergic channel activator (CHCA). Presented at 32nd Annual Meeting American College of Neuropsychopharmacology, December 13-17, Honolulu, HI
- Snodgrass J, Corwin J (1988) Pragmatics of recognition memory: application to dementia and amnesia. *J Exp Psychol [Gen]* 117:34-50
- Sunderland T, Tariot PN, Newhouse PA (1989) Differential responsiveness of mood, behavior, and cognition to cholinergic agents in elderly neuropsychiatric populations. *Brain Res Rev* 13:371-389
- Thompson D (1973) Repeated acquisition as a behavioral base line for studying drug effects. *J Pharmacol Exp Ther* 184:506-514
- Vitaliano PP (1982) Parametric statistical analysis of repeated measures experiments. *Psychoneuroendocrinology* 7:3-13
- Warburton DM, Rusted JM (1993) Cholinergic control of cognitive resources. *Neuropsychobiology* 28:43-46
- Wesnes K, Warburton D (1983) Smoking, nicotine, and human performance. *Pharmacol Ther* 21:189-208
- Whitehouse PJ, Martino AM, Antuona PG, Lowenstein PR, Coyle JT, Price DL, Kellar KJ (1986) Nicotinic acetylcholine binding sites in Alzheimer's disease. *Brain Res* 37:146-151
- Wilson AL, Langley LK, Monley J, Bauer T, Rottunda S, McFalls E, Kovera C, McCarten JR (1995) Nicotine patches in Alzheimer's disease: pilot study on learning, memory, and safety. *Pharmacol Biochem Behav* 51:509-514

(S)-3-Methyl-5-(1-Methyl-2-Pyrrolidinyl) Isoxazole (ABT 418): A Novel Cholinergic Ligand with Cognition-Enhancing and Anxiolytic Activities: I. *In Vitro* Characterization

STEPHEN P. ARNERIC, JAMES P. SULLIVAN, CLARK A. BRIGGS, DIANA DONNELLY-ROBERTS, DAVID J. ANDERSON, JOANNA L. RASZKIEWICZ, MARION L. HUGHES, EVELYN D. CADMAN, PAMELA ADAMS, DAVID S. GARVEY, JAMES T. WASICAK and MICHAEL WILLIAMS

Neuroscience Discovery, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois

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ABSTRACT

A diversity of nicotinic acetylcholine receptor (nAChR) subtypes has been identified in mammalian brain using recombinant DNA technology. Alterations in the activity of these acetylcholine-gated ion channels have been implicated in a number of central nervous system disorders including Alzheimer's disease (AD). The potential therapeutic usefulness of (–)-nicotine [(S)-3-(1-methyl-2-pyrrolidinyl) pyridine], the prototypic agonist at nAChRs, is severely limited by side effects that are the result of activation of both cholinergic and noncholinergic pathways in the central and peripheral nervous systems. This study sought to determine the *in vitro* selectivity of (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT 418), a novel analog of (–)-nicotine in which the pyridine ring was replaced with an isoxazole bioisotere, to activate nAChRs. ABT 418 was a potent inhibitor of [³H]-cytisine binding to nAChR in rat brain ($K_i = 3$ nM) but was inactive ($K_i > 10,000$ nM) in 37 other receptor/neurotransmitter-uptake/enzyme/transduction system binding assays, including those for α -bungarotoxin, muscarinic and 5-hydroxytryptamine₂ receptors. In PC12 cells, patch-clamp studies indicated that ABT 418 was an agonist with an EC_{50} value of 209 μ M, a potency to activate cholinergic channel currents some 4-fold less than that of (–)-

nicotine (52 μ M). Channel current responses elicited by ABT 418 were prevented by the cholinergic channel blocker, mecamylamine. ABT 418 was also approximately 10-fold less potent (EC_{50} value = 380 nM) than (–)-nicotine (40 nM) in increasing [³H]-dopamine release from rat striatal slices, an effect that was blocked by the nAChR antagonist, dihydro- β -erythroidine (10 μ M). In contrast, ABT 418 appeared equipotent with (–)-nicotine in enhancing ⁸⁶Rb⁺ flux from mouse thalamic synaptosomes. ABT 418 demonstrated an *in vitro* pharmacological profile of cholinergic channel activation that was robust at some nAChR, but not others. The reasons for this are unclear. However, a nAChR subtype selectivity may account for the *in vitro* potency differences of ABT 418 on various neurotransmitter systems, and the substantial separation between the cognitive enhancement/anxiolytic benefits, and the reduced central nervous system side-effect liabilities seen *in vivo*. ABT 418 represents the first neuronal nAChR ligand that differentiates the toxicities/liabilities and other negative aspects normally associated with (–)-nicotine from the potential pharmacological benefits of selective cholinergic channel activation.

Neuronal nAChRs in the CNS represent an expanding area of potential therapeutic opportunity driven by new findings in the molecular biology of the system (Changeux *et al.*, 1992; Deneris *et al.*, 1991; Sargent, 1993). The pharmacological properties and physiological function of these newly identified molecular targets remain, to a large extent, unknown primarily due to the lack of potent and selective pharmacological probes. Nonetheless, alterations in the activity of the acetylcholine-gated ion channels have been implicated in a number of CNS

disorders including AD (Arneric and Williams, 1994). Preliminary clinical data indicate that acutely administered (–)-nicotine, the prototypic agonist for nAChR, may be beneficial for the treatment of the deficits in attention and information processing associated with AD (Wesnes and Warburton, 1984; Newhouse *et al.*, 1988; Sahakian *et al.*, 1989; Jones *et al.*, 1992). Compounds that selectively interact with subtypes of nAChR to normalize CNS functions governed by this receptor family may, therefore, lead to more effective therapeutic agents (Arneric *et al.*, 1995).

An emerging diversity of *alpha* and *beta* nAChR subunits

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ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor(s); CNS, central nervous system; AD, Alzheimer's disease; 5-HT, 5-hydroxytryptamine (serotonin); α -Bgt, α -bungarotoxin; ABT 418, (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole hydrochloride; A-81754, (R)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole hydrochloride; DH β E, dihydro- β -erythroidine; PE1, polyethylenimine; DMEM, Dulbecco's modified Eagle's medium; ChCA, cholinergic channel activator(s).

have been identified in brain and autonomic ganglia using recombinant DNA technology (Deneris *et al.*, 1991). The nAChR found at the neuromuscular junction is a pentamer comprised of *alpha*, *beta*, *gamma*, *delta* and *epsilon* subunits that demonstrates developmental regulation of subunit composition (Mishina *et al.*, 1986; Changeux, 1990). However, the precise composition of the brain receptors is much less well characterized. Ten gene products *alpha* 2 through *alpha* 8, and *beta* 2 through *beta* 4 have been identified by the cloning of mammalian brain cDNA. Although many of the isolated subunits can form a functional nAChR when reconstituted in pairwise combinations in *Xenopus* oocytes (Sargent, 1993), the stoichiometry of these nAChR *in situ* has yet to be elucidated. At least seven potential neuronal nAChR subtypes have been identified in oocyte preparations (Luetje and Patrick, 1991; Courturier *et al.*, 1990) which have physiological and pharmacological properties that are similar to those native receptors found in various CNS preparations (Mulle *et al.*, 1991; Alkon and Albuquerque, 1993). The differing pharmacological profile of these functional nAChR suggests that they may represent unique targets for the development of CNS agents. Furthermore, the wide distribution of *alpha* 2, *alpha* 3, *alpha* 4, and *beta* 2 transcripts in the brain suggests that the neuronal nAChR are a neurotransmitter receptor superfamily that may be of global functional importance. The identification of physiological roles for these receptor subtypes presents a challenge and a therapeutic opportunity akin to the identification and development of selective ligands with demonstrated therapeutic utility for the ever expanding 5-HT-receptor superfamily (Zifa and Fillion, 1992).

One approach to identifying subtype selective ligands for nAChR is to evaluate the activity of compounds in adequate models of brain nAChR function—preferably those that contain native receptors found in various CNS preparations. Using receptor binding techniques, two major pharmacological subclasses of nAChR can be clearly delineated in mammalian brain (Clarke *et al.*, 1985; Marks *et al.*, 1986), *i.e.*, those that recognize α -Bgt with high affinity (α -Bgt nAChR) and those that do not (β -Bgt nAChR). α -Bgt nAChR do not display high-affinity binding to ligands like (–)-nicotine (Wonnacott, 1986), whereas β -Bgt nAChR do (Whiting and Lindstrom, 1986, 1988). The functional pharmacological extension of these receptor binding studies have been accomplished through the identification of *in vitro* preparations using electrophysiological (Pereira *et al.*, 1993; Alkon and Albuquerque, 1993; Schrattenholz *et al.*, 1993; Papke, 1993), and biochemical (Whiting and Lindstrom, 1986, 1988; Schoepfer *et al.*, 1990; Listerud *et al.*, 1991; Flores *et al.*, 1992; Grady *et al.*, 1992; Marks *et al.*, 1993) techniques that demonstrate selective responsivity for subtypes of nAChR. For example, more than 90% of nicotinic receptor binding with [³H]cytisine occurs at nAChR that contain the *alpha* 4/*beta* 2 subunits (Flores *et al.*, 1992). Correspondingly, this receptor subtype appears to mediate a flux of monovalent ions as measured by efflux of ⁸⁶Rb⁺ from mouse thalamic synaptosomes (Marks *et al.*, 1993). On the other hand, stimulation of [³H]-dopamine release in the striatum appears to be mediated by nAChR containing the *alpha* 3 subunit (Grady *et al.*, 1992). PC12 cells also contain the *alpha* 3, but little or no *alpha* 4, subunit isoforms (Rogers *et al.*, 1992), and can be readily evaluated using whole-cell patch-clamp techniques. In contrast, [¹²⁵I] α -Bgt labels nAChR formed by the *alpha* 7 subunit isoform found in brain (Courturier *et al.*, 1990), and the *alpha* 1 isoform

in the neuromuscular junction and in *Torpedo californica* (Changeux, 1990). Functionally, the *alpha* 7 homo-oligomer expressed in oocytes has a calcium permeability greater than neuromuscular receptors and, in some instances, greater than N-methyl-D-aspartate channels (Seguela *et al.*, 1993).

In this paper, the *in vitro* characterization of the novel nicotinic receptor ligand ABT 418 is described using tissue preparations that are recognized for their ability to express differentially subunit isoforms of nAChR and serve as bioassays to evaluate potentially selective activators of cholinergic channels. ABT 418 is a novel bioisostere of (–)-nicotine (Fig. 1; Garvey *et al.*, 1994) which, while sharing many of the positive CNS attributes of (–)-nicotine, has a reduced propensity to elicit the side effects that limit the usefulness of (–)-nicotine for the treatment of AD. In the accompanying paper (Decker *et al.*, 1994), the behavioral, cerebral circulatory, electroencephalographic and pharmacokinetic properties of this cholinergic ligand are described.

Materials and Methods

All animal studies were conducted in accord with American Association for the Accreditation of Laboratory Animal Care (AAALAC) procedures as approved by the Institutional Animal Care and Use Committee at Abbott Laboratories.

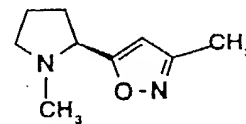
Compounds. ABT 418 and the *R*-isomer of ABT 418, A-81754 [(*R*)-3-methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole hydrochloride] were synthesized as described by Garvey *et al.* (1994). (–)-Nicotine di-(+)-tartrate salt, (+)-nicotine di-*p*-toluoyltartrate salt, mecamylamine hydrochloride, atropine and urethane were obtained from Sigma (St. Louis, MO). DH β E, MDL 72222 and diazepam were obtained from Research Biochemicals Int (Natick, MA). α -Bgt was obtained from Biotoxin Inc. (St. Cloud, FL). All radioligands were obtained from Du Pont-NEN (Boston, MA).

Stock solutions of ABT 418, A-81754, atropine and (–)-nicotine were prepared in distilled water. DH β E and MDL 72222 were prepared as 10 mM stock solutions in 100% dimethyl sulfoxide.

Receptor Binding

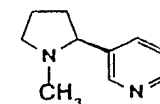
Membranes were prepared from whole rat brains (male Sprague-Dawley rats; 250–400 g; Sasco, Madison, WI) by the method of Enna and Snyder (1977). Brains were rapidly removed after decapitation homogenized in 15 vol of 0.32 M sucrose, and centrifuged at 1000 $\times g$ for 10 min at 4°C. The supernatants were removed and centrifuged at 20,000 $\times g$ for 20 min at 4°C. The resultant P₂ pellets were homogenized with a Polytron (10 sec, setting of 6) in ice-cold water and spun at 8,000 $\times g$ for 20 min. The supernatant and loose buffy coat were carefully removed and centrifuged at 40,000 $\times g$ for 20 min. The membrane pellet was washed with ice-cold H₂O and recentrifuged at 40,000 $\times g$ before storage at –80°C.

ABT 418



(*S*)-3-Methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole

(–)- Nicotine



(*S*)-3-(1-methyl-2-pyrrolidinyl) pyridine

Fig. 1. Structures of ABT 418 and (–)-nicotine.

[³H]Cytisine binding. [³H]Cytisine binding was performed using a modification of the method of Pabreza *et al.* (1991). Membrane enriched fractions were slowly thawed at 4°C and washed and resuspended in 30 vol of BSS-Tris buffer (BSS; 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 2 mM MgCl₂, and 50 mM Tris-Cl, pH 7.4, 4°C). Aliquots of protein (100–200 µg), 1.25 nM [³H]cytisine (30 Ci/mmol) and compounds to the final concentrations indicated were incubated in a final volume of 500 µl for 75 min at 4°C in duplicate. Nonspecific binding was determined in the presence of 10 µM (–)-nicotine. Bound radioactivity was separated under vacuum onto #32 glass fiber filters (Schleicher and Scheull, Keene, NH) using a Skatron filtration apparatus (Skatron, Sterling, VA). Filters were prerinsed with 0.5% PEI before sample filtration to reduce non-specific binding and were then rapidly rinsed with 4.5 ml of ice-cold BSS. Filters were counted in 3 ml of Ecolume (ICN, Costa Mesa, CA) at an efficiency of approximately 45% by conventional liquid scintillation counter (model LS5000 TD, Beckman Instruments Inc., Fullerton, CA).

[¹²⁵I]-α-Bgt binding. [¹²⁵I]-α-Bgt binding was determined in membranes prepared from whole rat brain and from *Torpedo Californica* electroplax.

α-Bgt binding to rat brain membranes was determined using a modification of the method of Marks *et al.* (1986). Rat brain membranes were resuspended in 15 vol of assay buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 20 mM HEPES, pH 7.5). Aliquots containing 200 µg of tissue were added to a reaction mixture containing 1.9 nM [¹²⁵I]-α-Bgt (106 Ci/mmol) and the indicated concentrations of ABT 418 or reference agents in triplicate. Nonspecific binding was determined in the presence of 1 µM unlabeled α-Bgt. Binding was conducted at 37°C for 3 hr. Bound radioactivity was isolated by rapid vacuum filtration onto #32 glass fiber filters (Schleicher and Scheull) using a Skatron filtration apparatus. Filters were prerinsed with 0.05% PEI and were then rapidly rinsed with 4.5 ml of ice-cold assay buffer. Radioactivity was measured in a gamma counter (model 5000, Beckman, Fullerton, CA).

A solid phase binding assay was used to measure the binding of [¹²⁵I]-α-Bgt (106 Ci/mmol) to the α-Bgt nAChR isolate from *Torpedo Californica* electroplax. The wells of a 96-well microtiter plate (Immulon Removawells Strips, Dynatech, Chantilly, VA) were coated with 0.5 µg of *Torpedo* membranes (ABS Inc., Wilmington, DE) in 50 mM NaHCO₃ buffer, pH 9.6, for 12 hr at 4°C. Wells were then washed twice with phosphate-buffered saline and quenched for 1 hr with 5% bovine serum albumin. [¹²⁵I]-α-Bgt (~1.9 nM/100 µl 10 mM phosphate buffer, pH 7.5/0.2% bovine serum albumin) was then added to the wells for 1 hr. For competition experiments, increasing concentrations of competitor (50 µl) were added to wells in triplicate followed immediately by 50 µl of [¹²⁵I]-α-Bgt and incubated for 1 hr. Nonspecific binding was determined in the presence of 1 µM α-Bgt. After incubation, wells were washed 5 times with phosphate-buffered saline. Individual wells were placed in vials and radioactivity measured in a gamma counter (model 5000, Beckman).

[³H]Oxotremorine-M binding assay. [³H]Oxotremorine-M (87 Ci/mmol) binding to the muscarinic cholinergic receptor was performed in 20 mM Na₂PO₄ buffer, pH 7.4 at 25°C for 45 min using a modification of the method of Birdsall *et al.* (1978). The assay mixture contained 100 µg of rat brain membranes per tube, 2 nM [³H]oxotremorine-M and the indicated concentrations of compounds in triplicate. Nonspecific binding was determined in the presence of 10 µM atropine. Radioactivity was isolated and radioactivity determined as described above.

[³H]Diazepam binding. [³H]Diazepam binding to the central benzodiazepine receptor present in rat brain cerebellar membranes was performed using a modification of the method of Falch *et al.* (1985). Fresh cerebella from adult Sprague-Dawley rats (Sasco, Madison, WI) were homogenized in 10 ml of ice-cold 100 mM Tris-citrate (pH 7.1) using a Polytron (setting 6 for 5 sec). The resultant homogenate was centrifuged at 20,000 × *g* for 10 min at 4°C and the pellet was washed five times by rehomogenization in ice-cold buffer and recentrifugation.

The final suspension was diluted to 0.5 mg/ml in 100 mM Tris-citrate (pH 7.1) with 150 mM NaCl.

The reaction mixture containing 0.8 nM [³H]diazepam (83.5 Ci/mmol), 100 µg of protein and various concentrations of compound in triplicate was incubated for 30 min at 30°C. Non-specific binding was determined in the presence of 3 µM diazepam. After incubation, the samples were collected on GF/C glass fiber filters (Whatman, Clifton, NJ) using a Skatron filtration apparatus and washed with ice-cold 150 mM Tris-citrate buffer. Filters were placed in 3 ml of Ecolume and radioactivity determined as described above.

[³H]GR-65630 binding. [³H]GR-65630 (62.5 Ci/mmol) binding to the 5HT₂ receptor was determined by the method of Hoyer and Neijt (1988). Cells of the N1E-115 mouse neuroblastoma clonal cell line (ATCC, Rockville, MD) were grown in DMEM supplemented with glutamine, 10% fetal bovine serum, 50 units penicillin/streptomycin, 1 mM sodium pyruvate, 1% nonessential amino acids and 2 mM L-glutamine (Gibco-BRL, Bethesda, MD). Cells were harvested in the presence of trypsin-EDTA (Gibco-BRL) to dissociate the cells from the flask surface followed by centrifugation of the medium at 600 × *g* for 6 min. The resultant cell pellets were homogenized using a Polytron (setting 6 for 5 sec) in 20 vol of 20 mM HEPES containing 50 mM NaCl (pH 7.5 at 25°C).

For competition experiments, aliquots of the homogenate (equivalent to 200,000 cells/tube) were incubated in the presence of 0.7 nM [³H]GR-65630 and the indicated concentrations of compound in triplicate for 30 min at 25°C. Nonspecific binding was determined in the presence of 10 µM MDL 72222. Bound radioactivity was isolated by rapid vacuum filtration onto GF/B glass fiber filters (Whatman) using a Skatron filtration apparatus. Filters were prerinsed with 0.05% PEI and were then rapidly rinsed with 5 ml of 20 mM HEPES containing 0.9% NaCl at 25°C. Filters were placed in 3 ml of Ecolume and radioactivity determined as described above.

Additional receptor selectivity binding studies. To assess further the selectivity of ABT 418 as an nAChR ligand, the compound was evaluated in a *PROFILE* receptor binding selectivity screen by NovaScreen (Hanover, MD) using standard receptor binding protocols (table 2). ABT 418 was tested at three concentrations (1, 100 and 10,000 nM) in duplicate in 35 binding assays for a number of neurotransmitter receptors, channel proteins, peptide factors, reuptake sites and second messenger systems.

Data analysis. In competition experiments, the drug concentration producing 50% inhibition (IC₅₀) of radioligand binding and the Hill coefficient (*n_H*) were determined from plots of (*B₀* – *B*)/*B₀* vs. log (concentration of drug), where *B₀* and *B* are specific binding in the absence and presence of competitor, respectively, using a four-parameter logistics program in RS/1 (Bolt Beranek and Newman Inc. Cambridge, MA). Inhibition constant (*K_i*) values were determined using the Cheng-Prusoff equation (*K_i* = IC₅₀/1 + [*L*]/*K_d*, where [*L*] = free radioligand concentration).

⁸⁶Rb⁺ Efflux:

The ability of ABT 418 and (–)-nicotine to activate ion channels was investigated by measuring efflux of the potassium ion analog ⁸⁶Rb⁺ from mouse thalamus using the methods of Marks *et al.* (1993).

Thalami were dissected from the brains of female C57 BL/61bg mice (60–90 days old; Institute for Behavioral Genetics, Boulder, CO) and homogenized in 10 vol (w/v) of ice-cold 0.32 M sucrose, 5 mM HEPES, pH 7.5 by hand using 16 strokes in a Teflon-glass homogenizer. The homogenate was diluted to 25 vol with ice-cold 0.32 M sucrose and centrifuged for 10 min at 1000 × *g* and the resulting supernatant recentrifuged for 20 min at 15,000 × *g* to yield the P₂ pellet. The P₂ fraction was resuspended in 8 vol of ice-cold perfusion buffer (140 mM NaCl, 1.5 mM KCl, 2 mM CaCl₂, 1 mM MgSO₄, 25 mM HEPES, 20 mM D-glucose, pH 7.5).

A P₂ fraction equivalent to two thalami was incubated for 30 min at 21°C in 35 ml of perfusion buffer containing 4 µCi of ⁸⁶Rb⁺ (35 Ci/mmol). At the end of the incubation period, tissue was harvested and separated from the incubation medium by filtration onto 6 mm diam-

eter glass fiber filters (type GC50, Microfiltration Systems, Dublin, CA) under gentle vacuum (-0.2 atm) followed by eight washes at room temperature with perfusion buffer. The filter containing the ^{86}Rb -loaded synaptosomes was placed on a 13-mm glass fiber filter (type GC50, Microfiltration Systems) mounted on the bottom half of a plastic filter holder (Swinney type, 13 mm, Gelman Sciences, Inc., Ann Arbor, MI) modified to reduce the dead volume beneath the filter platform by using 14-gauge stainless steel tubing inside the holder with epoxy cement. The filter containing the tissue was subsequently perfused continuously at 21°C . After an initial average wash period of 8 min, fractions were collected every 30 sec by using a Retraver II fraction collector (ISCO, Inc., Lincoln, NE). Exposure to ABT 418 and $(-)$ -nicotine occurred approximately 3 min into a 10-min collection period. In any experiment, five concentrations of each nicotinic receptor ligand were tested and the tissue on each filter stimulated only one time. $(-)$ -Nicotine ($10\ \mu\text{M}$) was included in each experiment as control to normalize values between experiments. Radioactivity was measured using a Packard Auto-Gamma counter (Packard, Naperville, IL) and the magnitude of the $^{86}\text{Rb}^+$ response amplitude calculated by determining the increase in radioactivity above the base line after stimulation of the tissue. The average base line underlying the peak was calculated by averaging the radioactivity present in the tubes immediately before and after the peak. Peak size was determined by subtracting the average base-line value from each fraction in the peak. To correct for differences in total tissue content and base-line release, the response was normalized by dividing by the amount of $^{86}\text{Rb}^+$ present in the tissue at the time of stimulation. Estimates of the EC_{50} values obtained for stimulation of $^{86}\text{Rb}^+$ efflux were calculated using Inplot (Graphpad, San Diego, CA), and relying on the assumption that the highest concentration of $(-)$ -nicotine used in this study elicited a near maximal ion flux (Marks *et al.*, 1993).

Striatal Dopamine Release

nAChR-evoked release of [ring-2,5,6- ^3H]dopamine ($24.4\ \text{Ci/mmol}$) was measured in superfused rat striatal slices. Striata were dissected from two male Sprague-Dawley rats per experiment and sliced $0.35 \times 0.25\ \text{mm}$ by a McIlwain Tissue Chopper (Brinkman Instrument Co., Westbury, NY). After two washes with Krebs-HEPES buffer ($137\ \text{mM NaCl}$, $4.7\ \text{mM KCl}$, $1\ \text{mM MgSO}_4$, $2.5\ \text{mM CaCl}_2$, $1.25\ \text{mM NaH}_2\text{PO}_4$, $10\ \text{mM glucose}$, $15\ \text{mM HEPES-NaOH}$, pH 7.4, containing $10\ \mu\text{M}$ pargyline and $10\ \mu\text{M}$ ascorbic acid), slices were preincubated for 10 min at 37°C under $95\%\ \text{O}_2/5\%\ \text{CO}_2$. After replacing the buffer, slices were labeled with $100\ \text{nM}$ [^3H]dopamine for 25 min in Krebs-HEPES at 37°C . Aliquots of slices were placed in 18 superfusion chambers of a Brandel SP2000 superfusion apparatus (Brandel, Gaithersburg, MD). After 47 min of washout at $0.5\ \text{ml/min}$, slices were exposed to agonist for 4 min. Antagonists, when present were introduced 4 min before and during agonist exposure. Collected 2-min fractions were counted in 5 ml of Ecolume. Tissue was recovered from superfusion chambers, solubilized with 1 ml of Solvable (DuPont-NEN) and counted in 15 ml of Ecolume.

Fractional release of [^3H]dopamine was calculated from radioactivity above baseline as a fraction of total radioactivity. Relative potencies were calculated using the release evoked by $100\ \text{nM}$ $(-)$ -nicotine as a standard. EC_{50} values were determined by nonlinear least squares regression analysis using InPlot.

Whole-Cell Channel Currents

Rat pheochromocytoma (PC12) cells were obtained from ATCC (Rockville, MD) and maintained in DMEM containing 10% heat-inactivated fetal calf serum and 5% heat-inactivated horse serum (37°C , $95\%\ \text{O}_2/5\%\ \text{CO}_2$). Nicotinic cholinergic responses were obtained from differentiated (neurite-bearing) cells after 4 to 7 days exposure to mouse nerve growth factor (NGF; Collaborative Biomedical Products, Bedford, MA). For this purpose, the undifferentiated cells were first plated onto poly-L-lysine-coated glass coverslips in plastic Petri dishes. After 20 min, differentiating medium (DMEM containing $100\ \text{ng/ml}$ NGF, 5% heat-inactivated fetal calf serum and 2.5% heat-inactivated horse

serum) was added and the cells were refed with this medium every 3 days.

The whole-cell patch-clamp technique was used to record voltage- and ligand-activated currents. A coverslip bearing the cells was transferred from culture dish to the recording chamber ($350\ \mu\text{l}$ vol) and superfused ($1\ \text{ml/min}$) at room temperature ($21\text{--}23^\circ\text{C}$) with an extracellular solution containing $150\ \text{mM NaCl}$, $2.8\ \text{mM KCl}$, $2.0\ \text{mM CaCl}_2$, $1.0\ \text{mM MgCl}_2$, $\geq 10\ \text{mM dextrose}$ and $10\ \text{mM Na-HEPES}$ buffer ($7.3\ \text{pH}$, $325\ \text{mOsm}$). The intracellular (recording pipette) solution contained $140\ \text{mM KCl}$, $1.0\ \text{mM CaCl}_2$, $2.0\ \text{mM MgCl}_2$, $11\ \text{mM K-EGTA}$, and $10\ \text{mM K-HEPES}$ buffer ($7.3\ \text{pH}$, $315\ \text{mOsm}$). Osmolarities were adjusted using dextrose such that the extracellular solution was $10\ \text{mOsm}$ hypertonic relative to the intracellular solution. Voltage-activated currents were monitored throughout the experiment to determine establishment and maintenance of the whole-cell recording configuration.

The cells were kept at a holding potential of $-60\ \text{mV}$ and cholinergic channel ligands dissolved in bathing solution were applied to the cells through computer-controlled U-tube flow reversal (Fenwick *et al.*, 1982) for a period of 5 sec. Each test compound was applied at least twice at each concentration in every cell, and applications were separated by $\geq 3\ \text{min}$ to allow for recovery from desensitization, washout of the bathing solution and re-equilibration of the U tube. Antagonists were applied to the bathing solution through superfusion for several minutes before application of both antagonist and putative agonist through flow-reversal. Data were acquired and quantified using an Axopatch 1B patch-clamp amplifier, a Tecmar Labmaster 125 KHz A/D system and pClamp software (Axon Instruments, Foster City, CA).

For quantifying dose-response relationships, each PC12 cell was exposed to several different concentrations of $(-)$ -nicotine or ABT 418 and the peak inward currents were measured. To correct for variation in PC12 cell responsiveness each response to various concentrations of $(-)$ -nicotine was normalized to the response to $100\ \mu\text{M}$ $(-)$ -nicotine obtained in the same cell; ABT 418 responses were normalized to $300\ \mu\text{M}$ ABT 418. Furthermore, the standards [$100\ \mu\text{M}$ $(-)$ -nicotine or $300\ \mu\text{M}$ ABT 418] were applied at the beginning, end and often middle of every experiment with each cell to evaluate changes in the response of the cell with time. The following equation was fit to the data using a nonlinear curve-fitting program (SigmaPlot, Jandel Scientific, San Rafael, CA):

$$I = I_{\text{max}} \times \frac{[A]^{n_H}}{\text{EC}_{50}^{n_H} + [A]^{n_H}}$$

where I is the observed current response, I_{max} is the maximal response, $[A]$ is the agonist concentration, EC_{50} is the agonist concentration that produces a half-maximal effect and n_H is the Hill coefficient.

Results

Receptor binding. Both ABT 418 and $(-)$ -nicotine competitively displaced [^3H]cytisine in a concentration-dependent manner with respective K_i values of $3.0 \pm 0.4\ \text{nM}$ ($n = 5$) and $1.0 \pm 0.1\ \text{nM}$ ($n = 3$; table 1; fig. 2). The concentration-response

TABLE 1

nAChR binding properties of ABT 418 and $(-)$ -nicotine

Values represent mean \pm S.E.M. Numbers in parentheses represent number of experiments.

	K_i (nM)		
	[^3H]cytisine	[^{125}I] α -Bungarotoxin	
	Rat Brain	Torpedo-Electroplax	Rat Brain
ABT 418	3.0 ± 0.4 (5)	$>100,000$ (3)	$>10,000$ (3)
A-81754	44 ± 12 (3)	$>10,000$ (3)	$>10,000$ (3)
$(-)$ -Nicotine	1.0 ± 0.1 (3)	$>100,000$ (3)	4000 ± 800 (3)

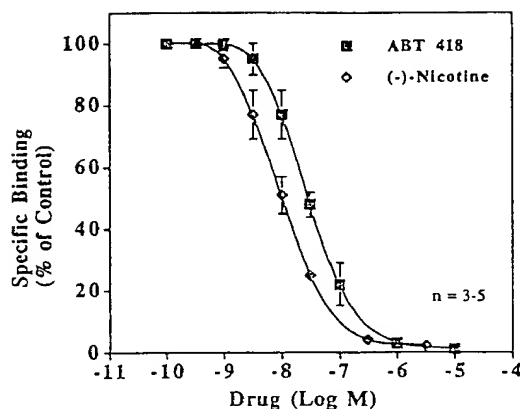


Fig. 2. Displacement of [^3H]cytisine binding by ABT 418 and (-)-nicotine. Rat brain membranes containing 100 to 200 μg of protein, 1.25 nM [^3H]cytisine and the indicated concentrations of drug were incubated in a total volume of 500 μl for 75 min at 4°C . ABT 418 and (-)-nicotine had K_i of 3 ± 0.4 and 1.0 ± 0.1 nM, respectively. Nonspecific binding was determined in the presence of 10 μM (-)-nicotine.

curve for ABT 418 was consistent with a single site competitive model ($n_H = 1.02 \pm 0.03$; $n = 5$) as was that for (-)-nicotine. The *R*-isomer of ABT 418, A-81754, was 12 times less potent ($K_i = 44$ nM) in displacing [^3H]cytisine whereas (-)-nicotine was some 3-fold more potent ($K_i = 1$ nM) than ABT 418 (table 1).

In contrast to its activity at the $\alpha 4 \beta 2$ nAChR subtype labeled by [^3H]cytisine (Flores *et al.*, 1992), ABT 418 was more than 3 orders of magnitude less potent (K_i value > 100 μM ; table 1) in displacing [^{125}I] α -Bgt bound to the nAChR subtype present in *Torpedo* electroplax which is similar to the neuromuscular α -Bgt nAChR. ABT 418 was similarly less potent ($K_i = > 10$ μM) than (-)-nicotine ($K_i = 4$ μM) in displacing [^{125}I] α -Bgt binding from the α -Bgt nAChR subtype present in rat brain membranes, *i.e.*, $\alpha 7$ (table 1). The *R*-isomer of ABT 418, A-81754, also had low potency ($K_i > 10$ μM) at the α -Bgt nAChRs present in both *Torpedo* and rat brain membranes (table 1).

ABT 418 also was examined in 38 other binding assays (table 2) and showed negligible affinity ($K_i > 10$ μM) for muscarinic, 5-HT $_3$ and the benzodiazepine receptors (table 2). ABT 418 had no significant effects on the binding of ligands, to other members of the ligand-gated ion channel superfamily, including γ -aminobutyric acid, benzodiazepine, N-methyl-D-aspartate, MK-801, quisqualate, kainate, L-, N- and T-calcium and potassium channel proteins; members of guanine nucleotide-binding protein-coupled receptor superfamily—adenosine A $_1$ and A $_{2a}$, $\alpha 1$, $\alpha 2$ and β adrenergic, 5HT $_1$, 5HT $_2$, histamine H $_1$, angiotensin-II, neurokinin-1 and -2, VIP, NGF; norepinephrine, 5-HT and dopamine uptake sites and second messenger systems including adenylate cyclase, protein kinase C and IP $_3$.

$^{86}\text{Rb}^+$ efflux from mouse thalamus. Both ABT 418 and (-)-nicotine elicited a concentration-dependent stimulation of efflux of $^{86}\text{Rb}^+$ from mouse thalamic synaptosomes (fig. 3) with responses of 0.7 ± 0.1 and $0.8 \pm 0.1\%$ of tissue content, respectively, for ABT 418 and (-)-nicotine at concentrations of 10 μM . The estimated EC_{50} values obtained for ABT 418 (0.5 ± 0.1 μM) and (-)-nicotine (0.7 ± 0.2 μM) were similar. ABT 418 appeared to be as potent and efficacious as (-)-nicotine in stimulating $^{86}\text{Rb}^+$ efflux. A-81754 was not examined

in this functional model. Because agonist-induced efflux of $^{86}\text{Rb}^+$ from mouse thalamic synaptosomes is thought to reflect the activation of the $\alpha 4 \beta 2$ subtype of nAChR (Marks *et al.*, 1993), ABT 418 appears to be at least as efficacious as (-)-nicotine at this subtype of nAChR.

[^3H]Dopamine release from rat striatal slices. ABT 418 was similar in efficacy to (-)-nicotine in stimulating the release of [^3H]dopamine from rat striatal slices. However (-)-nicotine ($\text{EC}_{50} = 40$ nM) was approximately 10-fold more potent than ABT 418 ($\text{EC}_{50} = 380$ nM) in evoking this response (fig. 4). A-81754 had an EC_{50} of greater than 1 μM in this model. The competitive nAChR antagonist, DH β E (10 μM) blocked the effects of ABT 418 (10 μM) in eliciting striatal [^3H]dopamine release by $89 \pm 10\%$ ($n = 3$). DH β E (10 μM) also blocked the effects of (-)-nicotine (10 μM) on [^3H]dopamine release (data not shown).

Cholinergic channel current responses in PC12 cells. The peak inward current responses to 100 μM (-)-nicotine ranged from -40 to -420 pA in 13 differentiated PC12 cells. ABT 418 (300 μM) elicited similar responses, ranging from -46 to -340 pA in another 4 cells (fig. 5). Desensitization of the response to each ligand was observed during the 5-sec application, as would be expected for nicotinic responses in these cells. Further, the nAChR channel blocker mecamylamine (10 μM) inhibited the response to ABT 418 (300 μM) by $81 \pm 3\%$ ($n = 3$).

Dose-response relationships were determined from 13 PC12 cells for (-)-nicotine, and from another four PC12 cells for ABT 418 (data not shown). The apparent EC_{50} value for ABT 418 was 209 ± 76 μM whereas that for (-)-nicotine was 52 ± 4 μM , indicating that ABT 418 is about 4-fold less potent than (-)-nicotine in activating cholinergic channels in PC12 cells.

Discussion

The data presented indicate that the newly synthesized isoxazole isostere of (-)-nicotine, ABT 418, is a potent cholinergic ligand with apparent selectivity for the neuronal, but not the neuromuscular, nAChR. ABT 418 is also less potent than (-)-nicotine in displacing [^{125}I] α -Bgt bound to rat brain membranes (table 1). In brain, α -Bgt is thought to label a protein corresponding to the $\alpha 7$ gene product (Couturier *et al.*, 1990) indicating that ABT 418 has less affinity for this subunit than does (-)-nicotine. This is important taking into consideration that approximately 50% of all nAChR in brain are of the α -Bgt nAChR subtype (*i.e.*, approximately 70 fmol/mg of protein for each, see Pabreza *et al.*, 1991; Marks *et al.*, 1986). Further selectivity is demonstrated by the lack of affinity (*i.e.*, $K_i > 10$ μM) for muscarinic receptors as well as at 36 other binding sites for differing classes of receptors, enzymes, uptake sites and second messengers. The stereoselective nature of the interaction of ABT 418 with the neuronal nAChR can be demonstrated by the reduced activity of the *R*-isomer of ABT 418, A-81754, in both the binding and functional test systems. Ligand binding studies with [^3H]ABT 418 indicate that it has a distribution in rat brain that differs from [^3H]cytisine and it does not elicit the same pattern of upregulation of nicotinic receptor binding sites after chronic administration as does (-)-nicotine (Schwartz and Kellar, 1983; Wonnacott, 1990; J.P. Sullivan, personal communication). Thus, the *in vitro* phar-

TABLE 2

In vitro receptor binding selectivity of ABT 418

Receptor	Ligand	K_i nM
Muscarinic	[³ H]oxotremorine-M	>10,000
Adenosine A ₁	[³ H]8-cyclopentyl-1,3-dipropylxanthine (CPX)	>10,000
Adenosine A _{2a}	[³ H]CGS 21680	>10,000
Alpha-1	[³ H]prazosin	>10,000
Alpha-2	[³ H]RX 781094	>10,000
Beta	[³ H]DHA	>10,000
Dopamine ₁	(+)-[¹²⁵ I]SCH23982	>10,000
Dopamine ₂	[³ H]Spiperone	>10,000
Quisqualate	[³ H]AMPA	>10,000
Kainate	[³ H]Kainic acid	>10,000
MK-801	[³ H]MK-801	>10,000
NMDA	[³ H]CGS 19755	>10,000
PCP	[³ H]TCP	>10,000
Glycine, Nonstrychnine	[³ H]Glycine	>10,000
Sigma	[³ H]Di(2-tolyl)guanidine (DTG)	>10,000
Glycine, strychnine	[³ H]Strychnine	>10,000
GABA _A	[³ H]GABA	>10,000
GABA _B	[³ H]GABA (+50 mM isoguvacine to block GABA _A)	>10,000
Benzodiazepine	[³ H]Flunitrazepam	>10,000
Serotonin ₁	[³ H]5-Hydroxytryptamine binoxalate	>10,000
Serotonin ₂	[³ H]Ketanserin	>10,000
Serotonin ₃	[³ H]GR65630	>10,000
Histamine ₁	[³ H]Pyrilamine	>10,000
Angiotensin II, central	[¹²⁵ I]Angiotensin II	>10,000
Neurokinin 1 (substance P)	[4,5- ³ H-Leu]Substance P	>10,000
Neurokinin 2 (substance K)	[¹²⁵ I]Neurokinin A	>10,000
Vasoactive intestinal peptide	[¹²⁵ I]Vasoactive Intestinal Peptide (VIP)	>10,000
Calcium channel (type N)	[¹²⁵ I] ω -Conotoxin	>10,000
Calcium channel (type T, L)	[³ H]Nitrendipine	>10,000
Chloride channel, TBOB	[³ H]TBOB	>10,000
Potassium channel, apamin	[¹²⁵ I]Apamin	>10,000
Nerve growth factor	[¹²⁵ I]Nerve growth factor	>10,000
Norepinephrine uptake	[³ H]Desmethylinipramine	>10,000
Serotonin uptake	[³ H]Citalopram	>10,000
Dopamine, cocaine site	[³ H]WIN 35,428	>10,000
Adenylate cyclase	[³ H]Forskolin	>10,000
Protein kinase C	[³ H]Phorbol ester dibutyrate (PDBU)	>10,000
Inositol triphosphate	[³ H]IP ₃	>10,000

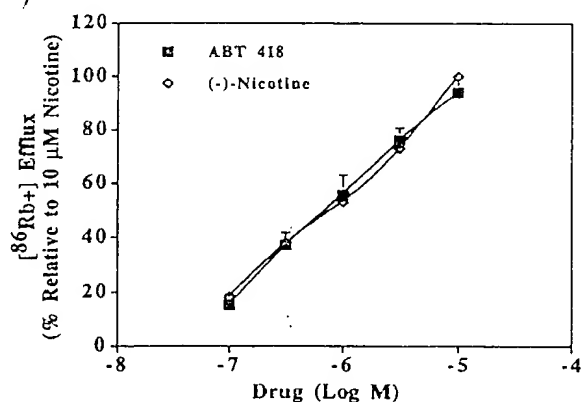


Fig. 3. Effect of ABT 418 and (-)-nicotine to stimulate (⁸⁶Rb⁺) efflux from mouse thalamic synaptosomes. P2 fractions from mouse thalamus that had been loaded with [⁸⁶Rb⁺] were exposed for 1 min to the concentrations of agonist indicated. Values are the mean \pm S.E.M.; $n = 3$ for ABT 418 and average of two separate experiments for (-)-nicotine. ABT 418 and (-)-nicotine had estimated EC₅₀ values of 0.5 ± 0.1 and 0.7 ± 0.2 μ M, respectively.

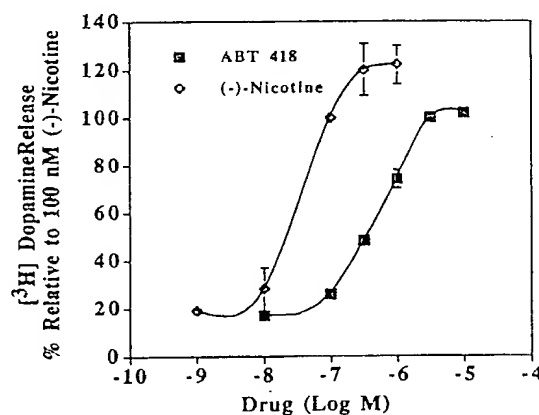


Fig. 4. Effect of ABT 418 and (-)-nicotine in stimulating the release of [³H]dopamine from rat striatal slices. Values are means \pm S.E.M.; ABT 418 and (-)-nicotine had EC₅₀s to evoke release of [³H]dopamine of 380 ± 45 and 40 ± 10 nM, respectively.

macodynamic effects of ABT 418 indicate that it has an activity profile that is substantially different than (-)-nicotine.

Mechanistically, ABT 418 can activate channel currents in PC12 cells, an effect that is prevented by the noncompetitive nAChR channel blocker, mecamylamine. However, because of

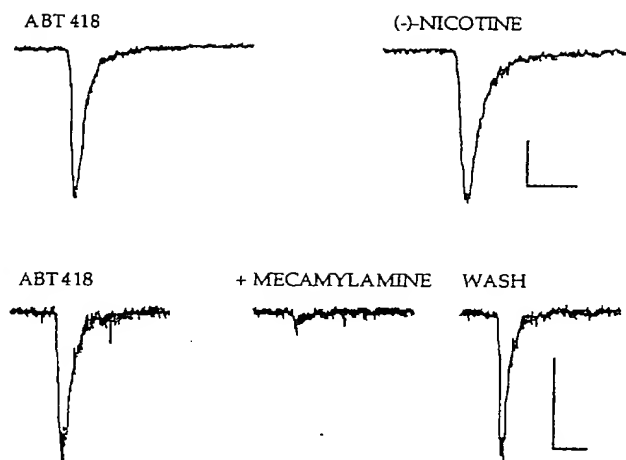


Fig. 5. Effect of ABT 418 and (-)-nicotine to elicit representative cholinergic channel currents in PC12 cells. In four nerve growth factor-differentiated PC12 cells, the inward current response to 300 μ M ABT 418 (upper left trace) ranged from -46 to -340 pA. The response to 100 μ M (-)-nicotine (upper right trace) similarly ranged from -40 to -420 pA in 13 other PC12 cells. The response to 300 μ M ABT 418 was inhibited reversibly by 10 μ M mecamylamine as shown for one cell in the lower three traces. Calibration lines represent 100 pA and 1 sec.

the noncompetitive nature of this antagonist (Gurney and Rang, 1984; Aracava *et al.*, 1987; Rapier *et al.*, 1990) further studies will be required to determine how ABT 418 is able to activate nAChR channel currents. The nAChR subunits associated with these cells are α 3, α 5, β 2, β 3 and β 4 (Rogers *et al.*, 1992), which are similar to those in sympathetic ganglia (Listerud *et al.*, 1991; Sargent, 1993). ABT 418 was about 4-fold less potent than (-)-nicotine in this PC12 test system, as well as in the rat superior cervical ganglion (C. A. Briggs, personal communication), suggesting that it has less affinity for nAChR expressing the α 3 subunit isoform. These data would predict that *in vivo* ABT 418 may have fewer effects related to activation of sympathetic ganglion than does (-)-nicotine.

In another preparation that has been suggested to involve α 3 subunit activation (Rapier *et al.*, 1990) ABT 418 stimulated the release of dopamine from striatal slices with an EC_{50} value of 380 nM, showing 10-fold lower potency than (-)-nicotine (EC_{50} = 40 nM). A-81754, the *R*-isomer of ABT 418, had an EC_{50} value of greater than 1 μ M in this system. Thus, the *S*- and *R*-isomers showed an approximately 14-fold difference in affinity when measured in the binding (table 1). This is similar to the stereoselectivity reported for the enantiomers of nicotine (Wonnacott *et al.*, 1990). In addition, the competitive nAChR antagonist DH β E (Williams and Robinson, 1984) antagonized the actions of ABT 418, suggesting that it may interact at the same site as (-)-nicotine to modulate dopamine release. It is thought that the addiction liabilities and locomotor stimulant effects of chronic exposure to compounds like (-)-nicotine are mediated by the release of dopamine elicited by the interaction of (-)-nicotine with nAChR on dopaminergic neurons (Clarke and Pert, 1985; Rapier *et al.*, 1990; Wonnacott *et al.*, 1990). The lower potency of ABT 418 on dopamine release compared to (-)-nicotine suggests that it may have less abuse potential than (-)-nicotine. This hypothesis is consistent with the finding that ABT 418 does not fully cross-discriminate with (-)-nicotine in rats and that it is 6-fold less potent than

(-)-nicotine to increase the firing of dopaminergic neurons in the ventral tegmental area of Tsai (Brioni *et al.*, 1994).

Changes in rubidium flux in the mouse thalamus are thought to result from activation of the putative α 4 β 2 form of the nAChR (Marks *et al.*, 1993). In this assay, ABT 418 and (-)-nicotine appeared to have equivalent activity (estimated EC_{50} values of 500 and 700 nM, respectively). It is noteworthy that none of the "classical" nAChR agonists that have been evaluated in this assay were both as potent and as efficacious as (-)-nicotine (Marks *et al.*, 1993). For example, the alkaloid (-)-cytisine, although twice as potent as (-)-nicotine, elicited a significantly lower maximum efflux. In contrast, acetylcholine, methylcarbamylcholine and 1,1-dimethyl-4-phenylpiperazine behaved as full agonists but were less potent than (-)-nicotine.

The concentrations of ABT 418 required to induce a functional response at the presumed α 4 β 2 subtype compared with those required to displace [3 H]cytisine in the binding assay differ by approximately 2 orders of magnitude. This quantitative difference may be due to the fact that stimulation of ion flux requires interaction with the receptors in their resting state whereas binding may measure the interaction of ligands with the high affinity desensitized state of the receptor (Lippello *et al.*, 1987).

The discrepancy in activity between ABT 418 and (-)-nicotine which is also seen *in vivo* (Decker *et al.*, 1994) in regard to the hypothermia, hypolocomotor activity, seizure activity, acute lethality and emetic liability associated with (-)-nicotine suggest that ABT 418 may preferentially interact with different subtypes of the nAChR. Alternatively, ABT 418 may act as a full agonist at certain receptor subtypes, e.g., α 4 β 2, while having partial agonist, or reduced activity at other receptor subtypes, an issue discussed further in regard to the *in vivo* actions of ABT 418 (Decker *et al.*, 1994).

It is unclear at which site on the nAChR in brain ABT 418 binds to elicit biological responses. At the neuromuscular junction there is evidence that the α 1 subunits contain elements for the binding of ACh (Changeaux, 1990). For neuronal nAChRs, there has been increasing evidence that alternative "channel activator" (Pereira *et al.*, 1993; Schrattenholz *et al.*, 1993) and allosteric modulatory sites exist (for review see Arneric *et al.*, 1995). Because ABT 418 has a functional profile that is different than (-)-nicotine, it is plausible that the pharmacological selectivity seen with this compound may arise from an interaction that is distinct from (-)-nicotine. Resolution of these possibilities will require more detailed pharmacological analysis of ABT 418 to be completed.

Research in the area of "nicotinic agonists" to date has been limited primarily to the biological evaluation of nicotine and other related naturally occurring alkaloids. The negative connotations associated with the recreational use of (-)-nicotine in tobacco products and the consequent negative impact on the patient health has tended to limit the research and development of nicotinic ligands as therapeutic entities. The discovery of multiple ChCA sites on nAChRs may be anticipated to renew interest in the nicotinic subclass of cholinergic receptors. ChCAs are by definition a broader pharmacological category of agents that may directly or allosterically activate one or more subtypes of the nAChR (Arneric and Williams, 1994; Lena and Changeaux, 1993). A potential therapeutic outcome of developing compounds that selectively interact with nAChR is that they do not necessarily elicit a side-effect profile like (-)-

nicotine. This has conceptual parallels with the use of the term "atypical anxiolytics" (Williams, 1989) to describe compounds like CGS 20625 which, while interacting with the central BDZ receptor complex, lacked the sedative, muscle relaxant and alcohol interactive properties associated with the classical BDZ, like diazepam. ABT 418 thus may represent the first of a new class of compounds termed ChCAs that selectively activate neuronal nAChR without eliciting the dose-limiting side effects typically observed with (-)-nicotine (Arneric and Williams, 1994; Decker *et al.*, 1994; Arneric *et al.*, 1994).

Since nicotinic receptor agonists are known to improve cognitive performance in experimental animals (Haroutunian *et al.*, 1985; Elrod *et al.*, 1988; Levin, 1992; Hodges *et al.*, 1992; Decker *et al.*, 1992) and in humans (Wesnes and Warburton, 1978, 1984; Newhouse *et al.*, 1988; Rusted and Eaton-Williams, 1991; Warburton, 1992) the availability of compounds like ABT 418 may represent a novel therapeutic approach for the amelioration of the cognitive and emotional disturbances accompanying AD and other related CNS disorders. Ongoing studies at the molecular level including receptor autoradiography using [³H] ABT 418 may be anticipated to lead to the identification of the discrete molecular targets through which it produces its many actions and permit the characterization of the ChCA recognition sites responsible for the potentially beneficial actions of this novel alkaloid.

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References

- ALKONDON, M. AND ALBUQUERQUE, E. X.: Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. *J. Pharmacol. Exp. Ther.* 265: 1455-1473, 1993.
- ARACAVA, Y., DESHPANDE, S. S., SWANSON, K. L., RAPOPORT, M., WONNACOTT, S., LUNT, G. G. AND ALBUQUERQUE, E. X.: Nicotinic acetylcholine receptors in cultured neurones from the hippocampus and brain stem of the rat characterized by single channel recording. *FEBS Lett.* 222: 63-70, 1987.
- ARNERIC, S. P., SULLIVAN, J. P. AND WILLIAMS, M.: Neuronal nicotinic acetylcholine receptors: Novel targets for CNS therapeutics. In *Psychopharmacology: The Generation of Progress*, ed. by F. E. Bloom and D. J. Kupfer, Raven Press, New York, in press, 1995.
- ARNERIC, S. P. AND WILLIAMS, M.: Nicotinic agonists in Alzheimer's disease: Does the molecular diversity of nicotine receptors offer the opportunity for developing CNS selective cholinergic channel activators? In *Recent Advances in the Treatment of Neurodegenerative Disorders and Cognitive Function*, vol. 7, ed. by G. Racagni, N. Brunello, S. Z. Langer, pp. 58-70, Karger, Basel, 1994.
- BIRDSALL, N. J. M., BURGEN, A. S. V. AND HULME, E. C.: The binding of agonists to brain muscarinic receptors. *Mol. Pharmacol.* 14: 723-736, 1978.
- BRIONI, J. D., KIM, D. J. B., BRODIE, M. S., DECKER, M. W. AND ARNERIC, S. P.: ABT-418: Discriminative stimulus properties and effect on ventral tegmental cell activity. *Psychopharmacology*, in press, 1994.
- CHANGEUX, J.-P.: Functional architecture and dynamics of the nicotinic acetylcholine receptor: An allosteric ligand-gated ion channel. *Fidia Res. Found. Neurosci. Found. Lect.* 4: 21-168, 1990.
- CHANGEUX, J.-P., GALZI, J.-L., DEVILLERS-THIERY, A. AND BERTRAND, D.: The functional architecture of the acetylcholine nicotinic receptor explored by affinity labeling and site directed mutagenesis. *Quart. Rev. Biophys.* 25: 395-432, 1992.
- CLARKE, P. B. S. AND PERT, A.: Autoradiographic evidence for nicotinic receptors on nigrostriatal and mesolimbic dopaminergic neurons. *Brain Res.* 348: 355-358, 1985.
- CLARKE, P. B. S., SCHWARTZ, R. D., PAUL, S. M., PERT, C. B. AND PERT, A.: Nicotinic binding in brain: autoradiographic comparison of [³H]acetylcholine, [³H]nicotine, and [¹²⁵I]- α -bungarotoxin. *J. Neurosci.* 5: 1307-1315, 1985.
- COUTURIER, S., BERTRAND, D., MATTER, J.-M., HERNANDEZ, M.-C., BERTRAND, S., *et al.*: A neuronal nicotinic acetylcholine receptor subunit ($\alpha 7$) is developmentally regulated and forms a homo-oligomeric channel blocked by α -BTX. *Neuron* 5: 847-856, 1990.
- MICHAEL W. DECKER, JORGE D. BRIONI, JAMES P. SULLIVAN, MICHAEL J. BUCKLEY, RICHARD J. RADEK, JOANNA L. RASZKIEWICZ, CHAE HEE KANG, DAVID J. B. KIM, WILLIAM J. GIARDINA, JAMES WASICAK, DAVID S. GARVEY, MICHAEL WILLIAMS AND STEPHEN P. ARNERIC (S)-3-Methyl-5-(1-Methyl-2-Pyrrolidinyl)isoxazole (ABT 418): A Novel Cholinergic Ligand with Cognition-Enhancing and Anxiolytic Activities: II. *In Vivo* Characterization 270: 319-328, 1994.
- DECKER, M. W., MAJCHRAK, M. J. AND ANDERSON, D. J.: Effects of nicotine on spatial memory deficits in rats with septal lesions. *Brain Res.* 572: 281-285, 1992.
- DENERIS, E. S., CONNOLLY, J., ROGERS, S. W. AND DUVOISIN, R.: Pharmacological and functional diversity of neuronal nicotinic acetylcholine receptors. *Trends Pharmacol. Sci.* 12: 34-40, 1991.
- ELROD, K., BUCCAFUSCO, J. J. AND JACKSON, W. J.: Nicotine enhances delayed matching-to-sample performance in primates. *Life Sci.* 43: 277-287, 1988.
- ENNA, S. J. AND SNYDER, S. H.: Properties of γ -aminobutyric acid receptor binding in rat brain synaptic membrane fractions. *Brain Res.* 100: 81-87, 1977.
- FALCH, E., JACOBSEN, P., KROGSGAARD-LARSEN, P. AND CURTIS, D. R.: GABA-mimetic activity and effects on diazepam binding of aminosulphonic acids structurally related to piperidine-4-sulphonic acid. *J. Neurochem.* 44: 68-75, 1985.
- FENWICK, E. M., MARTY, A. AND NEHER, E.: A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *J. Physiol. (Lond.)* 331: 577-597, 1982.
- FLORES, C. M., ROGERS, S. W., PABREZA, L. A., WOLFE, B. AND KELLAR, K. J.: A subtype of nicotinic cholinergic receptor is composed of $\alpha 4$ and $\beta 2$ subunits and is upregulated by chronic nicotine treatment. *Mol. Pharmacol.* 41: 31-37, 1992.
- GARVEY, D. S., WASICAK, J., DECKER, M. W., BRIONI, J. D., BUCKLEY, M. J., SULLIVAN, J. P., CARRERA, G. M., HOLLADAY, M. H., ARNERIC, S. P. AND WILLIAMS, M.: Novel isoxazoles which interact with brain cholinergic channel receptors that have intrinsic cognitive enhancing and anxiolytic activities. *J. Med. Chem.* 37: 1055-1059, 1994.
- GRADY, S., MARKS, M. J., WONNACOTT, S. AND COLLINS, A. C.: Characterization of nicotinic receptor mediated [³H]dopamine release from synaptosomes prepared from mouse striatum. *J. Neurochem.* 59: 848-856, 1992.
- GURNEY, A. M. AND RANG, H. P.: The channel blocking action of methonium compounds on rat submandibular ganglion cells. *Br. J. Pharmacol.* 82: 623-642, 1984.
- HAROUTUNIAN, V., BARNES, E. AND DAVIS, K. L.: Cholinergic modulation of memory in rats. *Psychopharmacology* 87: 266-271, 1985.
- HODGES, H., SINDEN, J., TURNER, J. J., NETTO, C. A., SOWINSKI, P. AND GRAY, J. A.: Nicotine as a tool to characterize the role of the basal forebrain cholinergic projection system in cognition. In *The Biology of Nicotine: Current Research Issues*, ed. by P. M. Lippiello, A. C. Collins, J. A. Gray and J. H. Robinson, pp. 157-182, New York, Raven Press, 1992.
- HOVER, D. AND NEIJT, H. C.: Identification of serotonin 5-HT₂ recognition sites in membranes of N1E-115 neuroblastoma cells by radioligand binding. *Mol. Pharmacol.* 33: 303-309, 1988.
- JONES, G. M. M., SAHAKIAN, B. J., LEVY, R., WARBURTON, D. M. AND GRAY, J. A.: Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. *Psychopharmacology* 108: 485-494, 1992.
- LENA, C. AND CHANGEUX, J.-P.: Allosteric modulation of the nicotinic acetylcholine receptor. *Trends Pharmacol. Sci.* 16: 181-186, 1993.
- LEVIN, E. D.: Nicotinic systems in cognitive function. *Psychopharmacology* 108: 417-431, 1992.
- LIPPIELLO, P. M., SEARS, S. B. AND FERNANDES, K. G.: Kinetics and mechanism of action of L-[³H] nicotine binding to putative high affinity receptor sites in rat brain. *Mol. Pharmacol.* 31: 392-400, 1987.
- LISTERUD, M., BRUSSAARD, A. B., DEVAY, P., COLMAN, D. R. AND ROLE, L. W.: Functional contribution of neuronal AChR subunits revealed by antisense oligonucleotides. *Science (Washington, DC)* 254: 1518-1521, 1991.
- LUETJE, C. W. AND PATRICK, J.: Both α - and β -subunits contribute to the agonist sensitivity of neuronal nicotinic acetylcholine receptors. *J. Neurosci.* 11: 837-845, 1991.
- MARKS, M. J., FARNHAM, D. A., GRADY, S. R. AND COLLINS, A. C.: Nicotinic receptor function determined by stimulation of nifedipine efflux from mouse brain synaptosomes. *J. Pharmacol. Exp. Ther.* 264: 542-552, 1993.
- MARKS, M. J., STITZEL, J. A., ROMM, E., WEHNER, J. M. AND COLLINS, A. C.: Nicotinic binding sites in rat and mouse brain: comparison of acetylcholine, nicotine and α -bungarotoxin. *J. Pharmacol. Exp. Ther.* 30: 427-436, 1986.
- MISHINA, M., TAKAI, T., IMOTO, K., NODA, M., TAKAHASHI, T., NUMA, S., METHFESSEL, C. AND SAKMANN, B.: Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. *Nature (Lond.)* 321: 406-411, 1986.
- MULLE, C., VIDAL, C., BENOIT, P. AND CHANGEUX, J.-P.: Existence of different subtypes of nicotinic acetylcholine receptors in the rat habenulo-interpeduncular system. *J. Neurosci.* 11: 2588-2597, 1991.
- NEWHOUSE, P. A., SUNDERLAND, T., TARIOT, P. N., BLUMHARDT, C. L., WEINGARTNER, H., MELLOW, A. AND MURPHY, D. L.: Intravenous nicotine in Alzheimer's disease: A pilot study. *Psychopharmacology* 95: 171-175, 1988.
- PABREZA, L. A., DHAWAN, S. AND KELLAR, K. J.: [³H]Cytisine binding to nicotinic cholinergic receptors in brain. *Mol. Pharmacol.* 39: 9-12, 1991.
- PAPKE, R. L.: The kinetic properties of neuronal nicotinic receptors: Genetic basis of functional diversity. *Prog. Neurobiol.* 41: 509-531, 1993.
- PEREIRA, E. F. R., REINHARDT-MAELICKE, S., SCHRATTENHOLZ, A., MAELICKE,

- A. AND ALBUQUERQUE, E. X.: Identification and functional characterization of a new agonist site on nicotinic acetylcholine receptors of cultured hippocampal neurons. *J. Pharmacol. Exp. Ther.* 265: 1474-1491, 1993.
- RAPIER, C., LUNT, G. G. AND WONNACOTT, S.: Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: concentration dependence and repetitive stimulation. *J. Neurochem.* 50: 1123-1130, 1990.
- ROGERS, S. W., MANDELZYS, A., DENERIS, E. S., COOPER, E. AND HEINEMANN, S.: The expression of nicotinic acetylcholine receptors by PC12 cells treated with NGF. *J. Neurosci.* 12: 4611-4623, 1992.
- RUSTED, J. AND EATON-WILLIAMS, P.: Distinguishing between attentional and amnesic effects in information processing: The separate and combined effects of scopolamine and nicotine on verbal free recall. *Psychopharmacology* 104: 363-366, 1991.
- SAHAKIAN, B., JONES, G., LEVY, R., GRAY, J. AND WARBURTON, D.: The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of the Alzheimer type. *Br. J. of Psychiatry* 154: 797-800, 1989.
- SARGENT, P. B.: The diversity of neuronal nicotinic acetylcholine receptors. *Ann. Rev. Neurosci.* 16: 403-443, 1993.
- SCHOEPPER, R., CONROY, W. G., WHITING, P., GORE, M. AND LINDSTROM, J.: Brain α -bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. *Neuron* 5: 35-48, 1990.
- SCHRATTENHOLZ, A., COBAN, T., SCHRODER, B., OKONJO, K. O., KUHLMANN, J., PEREIRA, E. F., ALBUQUERQUE, E. X. AND MAELICKE, A.: Biochemical characterization of a novel channel activating site on nicotinic acetylcholine receptors. *J. Receptor Res.* 13: 393-412, 1993.
- SCHWARTZ, R. D. AND KELLAR, K. J.: Nicotinic cholinergic binding sites in the brain: *In vivo* regulation. *Science* (Washington, DC) 220: 214-216, 1983.
- SEGUELA, P., WADICHE, J., DINELEY-MILLER, K., DANI, J. A. AND PATRICK, J. W.: Molecular cloning, functional properties and distribution of rat brain $\alpha 7$. A nicotinic cation channel highly permeable to calcium. *J. Neurosci.* 13: 596-604, 1993.
- WARBURTON, D. M.: Nicotine as a cognitive enhancer. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 16: 181-191, 1992.
- WESNES, K. AND WARBURTON, D. M.: The effects of cigarette smoking and nicotine tablets upon human attention. *In* *Smoking Behavior: Physiological and Psychological Influences*, ed. by R. E. Thornton, pp. 131-147, Churchill-Livingston, London, 1978.
- WESNES, K. AND WARBURTON, D. M.: Effects of scopolamine and nicotine on human rapid information processing performance. *Psychopharmacology* 82: 147-150, 1984.
- WHITING, P. AND LINDSTROM, J.: Pharmacological properties of immuno-isolated neuronal nicotinic receptors. *J. Neurosci.* 6: 3061-3069, 1986.
- WHITING, P. AND LINDSTROM, J.: Characterization of bovine and human neuronal nicotinic acetylcholine receptors using monoclonal antibodies. *J. Neurosci.* 8: 3395-3404, 1988.
- WILLIAMS, M.: Antianxiety agents: A historical perspective. *In* *Current and Future Trends in Anticonvulsant, Anxiety and Stroke Therapy*, ed. by B. S. Meldrum and M. Williams, Alan R. Liss, New York, pp. 477-482, 1989.
- WILLIAMS, M. AND ROBINSON, J. L.: Binding of the nicotinic cholinergic antagonist, dihydro-beta-erythroidine (DBE) to rat brain tissue. *J. Neurosci.* 4: 2906-2911, 1984.
- WONNACOTT, S.: α -Bungarotoxin binds to low-affinity binding sites in rat brain. *J. Neurochem.* 47: 1706-1712, 1986.
- WONNACOTT, S.: The paradox of nicotinic acetylcholine receptor upregulation by nicotine. *Trends Pharmacol. Sci.* 11: 216-219, 1990.
- WONNACOTT, S., IRONS, J., RAPIER, C., THORNE, B. AND LUNT, G. G.: Presynaptic modulation of transmitter release by nicotinic receptors. *In* *Progress in Brain Research*, ed. by A. Nordberg, K. Fuxe, B. Holmstedt and A. Sundwall, pp. 157-163, Amsterdam, Elsevier, 1990.
- ZIFA, E. AND FILLION, G.: 5-Hydroxytryptamine receptors. *Pharmacol. Rev.* 44: 401-458, 1992.

Send reprint requests to: Stephen P. Americ, Neuroscience, D 47W, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064-3500.

(S)-3-Methyl-5-(1-Methyl-2-Pyrrolidinyl)Isoxazole (ABT 418): A Novel Cholinergic Ligand with Cognition-Enhancing and Anxiolytic Activities: II. *In Vivo* Characterization

MICHAEL W. DECKER, JORGE D. BRIONI, JAMES P. SULLIVAN, MICHAEL J. BUCKLEY, RICHARD J. RADEK, JOANNA L. RASZKIEWICZ, CHAE HEE KANG, DAVID J. B. KIM, WILLIAM J. GIARDINA, JAMES T. WASICAK, DAVID S. GARVEY, MICHAEL WILLIAMS and STEPHEN P. ARNERIC

Neuroscience Discovery (M.W.D., J.D.B., J.P.S., M.J.B., R.J.R., J.L.R., C.H.K., D.J.B.K., J.W., O.S.G., M.W., S.P.A.) and Drug Design and Delivery (W.J.G.), Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois

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ABSTRACT

(S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole (ABT 418), an isoxazole analog of (–)-nicotine, is a potent agonist at the α -4/ β -2 subtype of neuronal nicotinic acetylcholine receptor (nAChR) that exists in mammalian brain (Arneric *et al.*, 1994). Compared to (–)-nicotine, ABT 418 has reduced potency to interact with the subunit isoforms of nAChR found in sympathetic ganglia, and it does not compete for α -bungarotoxin binding sites in brain or at the neuromuscular junction. ABT 418 [minimum effective dose (MED), 0.062 μ mol/kg i.p.] was 10-fold more potent in improving retention of avoidance learning in normal mice than (–)-nicotine, whereas the (R)-enantiomer of ABT 418, A-81754, was inactive. The memory-enhancing effect of ABT 418 was prevented by the nAChR channel blocker, mecamylamine. In the elevated plus-maze model of anxiety, ABT 418 (MED, 0.19 μ mol/kg i.p.) increased open-arm exploration in mice, as previously shown for (–)-nicotine (MED, 0.62 μ mol/kg i.p.). A-81754, did not have anxiolytic-like effects in this test. Unlike the classical anxiolytic, diazepam, ABT 418 did not impair rotorod performance in the dose range where beneficial effects occurred. In rats, ABT 418 (MED, 0.002 μ mol/kg i.v.) was remarkably potent in enhancing basal forebrain-elicited increases in cortical

cerebral blood flow, whereas resting cerebral blood flow was unaffected. Free running cortical electroencephalography in rats was unaffected by ABT 418 at a dose of 1.9 μ mol/kg i.p., whereas the same dose of (–)-nicotine caused cortical activation (decreased power in the 1–13 Hz range and increased power in the 25–50 Hz range). Whereas ABT 418 was approximately 3- to 10-fold more potent than (–)-nicotine in memory enhancement and anxiolytic test paradigms, the compound had less emetic liability in dogs as compared to (–)-nicotine, and was less potent than (–)-nicotine in eliciting hypothermia, seizures, death and reduction of locomotor activity in mice. The measured pharmacokinetic or brain disposition properties of ABT 418 in rats did not account for the observed enhancement in efficacy with reduced toxicity as compared to (–)-nicotine. The potent cognitive-enhancing and anxiolytic properties obtained for ABT 418 in animal models without eliciting significant side effects suggest that this ligand is a selective activator of cholinergic channel-mediated behaviors. Thus, ABT 418 may represent a novel, safe and effective treatment of the cognitive and emotional dysfunctions associated with Alzheimer's disease.

AD is associated with a loss of learning and memory abilities, attentional deficits, anxiety, agitation and depression (Perry *et al.*, 1978). Other impaired brain functions include reductions in cerebral blood flow, cerebral glucose utilization and abnormal EEG (Dastur, 1985; Petit *et al.*, 1993). Topographically these impaired brain functions correspond to the loss of the cholinergic innervation arising from the basal forebrain (Coyle *et al.*, 1983) and to substantial reductions in neuronal nAChRs (Whitehouse *et al.*, 1981, 1986).

Although the prevailing dogma suggests that muscarinic cholinergic receptors mediate the primary effects of central cholinergic transmission on cognitive performance (Bartus *et al.*, 1982, 1985) and cerebral vasodilation (Lee, 1982; Pinard, 1989; Hamel and Estrada, 1989), replacement therapy targeting muscarinic receptors has not been a fruitful approach to the amelioration of AD symptomatology (Arneric and Williams, 1993; Williams, 1993). However, pilot clinical data indicate that acutely administered (–)-nicotine, the prototypic agonist for nAChR, may be beneficial for the treatment of the deficits in attention and information processing associated with AD (Newhouse *et al.*, 1988; Sahakian *et al.*, 1989; Jones *et al.*, 1992).

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ABBREVIATIONS: ACh, acetylcholine; AD, Alzheimer's disease; EEG electroencephalography; nAChR, nicotinic acetylcholine receptor; CNS, central nervous system; ABT 418, (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole; A-81754, (R)-3-methyl-5-(1-methyl-2-pyrrolidinyl)isoxazole hydrochloride; ANOVA, analysis of variance.

Extensive evidence exists to indicate that activation of nAChR improves cognitive performance and improves cerebral functions in experimental animals and normal humans. (–)-Nicotine enhances cognitive function in normal rats (Levin *et al.*, 1990; Levin, 1992) and attenuates memory deficits produced by destruction of cholinergic input to the cortex and hippocampus (Tilson *et al.*, 1988; Decker *et al.*, 1992; Hodges *et al.*, 1992), an effect shared by some other nAChR agonists (Decker *et al.*, 1993; Meyer *et al.*, 1994). In addition, (–)-nicotine improves short-term memory performance in both young and aged monkeys (Elrod *et al.*, 1988; Buccafusco and Jackson, 1991). The involvement of nicotinic neurotransmission in cognitive function processes is further substantiated by observed deficits in cognitive performance after administration of mecamylamine, a nAChR channel blocker, to rodents (Oliverio, 1966; Levin *et al.*, 1987; Riekkinen *et al.*, 1990; Decker and Majchrzak, 1992), monkeys (Elrod *et al.*, 1988) and humans (Newhouse *et al.*, 1992). Moreover, the characteristic cortical cerebral blood flow abnormality associated with AD reflects nAChR deficits. Specifically, it has been demonstrated that mecamylamine, but not the muscarinic antagonist, scopolamine, reduces resting cortical cerebral blood flow in the parietotemporal cortex of humans (Gitelman and Prohovnik, 1992), the area most consistently implicated in functional brain imaging of AD patients (Prohovnik *et al.*, 1988; Risberg *et al.*, 1990; Geaney *et al.*, 1990; Heiss *et al.*, 1990). Reduced nicotinically mediated cerebral blood flow responses would, thus, be consistent with the loss of nAChR reported in several cortical regions using various labeling techniques (Whitehouse *et al.*, 1986; Araujo *et al.*, 1988; Schroder *et al.*, 1991; Aubert *et al.*, 1992), as well as a report suggesting a loss of basal forebrain nAChR population in AD (Shimohama *et al.*, 1986).

(–)-Nicotine, however, has limited utility as a therapeutic agent for AD because of its dose-limiting side effects in humans, which are primarily gastrointestinal (*e.g.*, nausea, abdominal pain) and cardiac (*e.g.*, increased catecholamine released resulting in tachycardia, peripheral vasoconstriction and elevated blood pressure) in nature. In an aged patient population these latter effects may result in more serious complications (Benowitz, 1992), especially in patients with pre-existing arrhythmias or angina pectoris. Compounds that selectively interact with subtypes of nAChR to normalize CNS functions modulated by this receptor superfamily may, therefore, offer the potential for more effective therapeutic agents.

ABT 418 is a novel bioisostere of (–)-nicotine (Arneric *et al.*, 1994; Garvey *et al.*, 1994) that selectively activates mammalian brain nAChRs in a manner that suggests interactions with a subpopulation of non- α -bungarotoxin sensitive nAChRs. A summary of some of the *in vitro* findings with this compound from Arneric *et al.* (1994) is presented in table 1. The present study demonstrates *in vivo* that ABT 418, while sharing many of the positive CNS attributes of (–)-nicotine, has a reduced propensity to elicit the side effects that limit the potential of (–)-nicotine for the safe treatment of AD.

Materials and Methods

All animal studies were conducted in accord with American Association for the Accreditation of Laboratory Animal Care (AAALAC) procedures as approved by the Institutional Animal Care and Use Committee at Abbott Laboratories.

TABLE 1

Overview of the *in vitro* pharmacological properties of ABT 418 and the (R)-enantiomer, A-81754, compared to (–)-nicotine

Assay Procedure	ABT 418 (S-form)	A-81754 (R-form)	(–)-Nicotine (S-form)
nAChR binding (K_i , nM)			
[³ H]cytisine	3 ± 0.4	44 ± 12	1 ± 0.1
[¹²⁵ I] α -bungarotoxin	>10,000	>10,000	4000 ± 800
PC12 cell current activation (EC ₅₀ , μ M)	209 ± 76	ND*	52 ± 4
⁸⁶ Rb ⁺ efflux from thalamic synaptosomes (estimated EC ₅₀ , μ M)	0.5 ± 0.1	ND*	0.7 ± 0.2
[³ H]Dopamine release from striatal slices (EC ₅₀ , μ M)	0.38 ± 0.05	>1	0.04 ± 0.01

* ND, not determined.

Compounds

ABT 418 and its (R)-enantiomer, A-81754, were synthesized as described (Garvey *et al.*, 1994). (–)-Nicotine di-(+)-tartrate salt, (+)-nicotine di-*p*-toluoyltartrate salt, mecamylamine hydrochloride, diazepam and urethane were obtained from Sigma Chemical Company (St. Louis, MO). Morphine sulfate was obtained from Mallinckrodt (St. Louis, MO). These compounds were dissolved in sterile 0.9% saline and injected in a volume of 10 ml/kg for mice and 1 ml/kg for rats. Fresh solutions were prepared each day. Curare injectable was purchased from Abbott Laboratories (Abbott Park, IL). Halothane was purchased from Halocarbon Laboratories, Inc. (North Augusta, SC).

Behavioral Studies

Male, CD-1 mice (Charles River, Portage, MI) weighing approximately 30 g were used in the behavioral experiments. The mice were housed 14 to a cage in a climate-controlled environment with free access to food and water. A 12:12-hr light/dark cycle (lights on at 6:00 A.M.) was used with testing conducted during the light portion of the cycle.

Inhibitory (passive) avoidance: Inhibitory avoidance training was conducted using an automated avoidance training system (Gemini, San Diego Instruments, San Diego, CA). ABT 418 and A-81754 were administered i.p. 15 min before the beginning of the training session. When used, the nAChR channel blocker, mecamylamine, or saline was administered 5 min before the ABT 418. Training was initiated by placing the mouse in a 13 × 14 × 13 (L × W × H) cm, brightly lit chamber. After a delay of 10 sec, a guillotine door leading to a larger (21 × 25 × 17 cm), darkened chamber automatically opened. When the mouse crossed completely into the dark chamber, the door was closed and a scrambled, constant current (0.3 mA) footshock was delivered through a grid floor for 2 sec. After the termination of the footshock the mouse was removed from the apparatus and returned to its home cage. Retention of the training experience was assessed 24 hr later by placing the mouse in the brightly lit compartment and measuring its latency to enter the dark compartment. On this retention test day, the trial was terminated after 180 sec and animals not crossing into the dark chamber before the end of the session were assigned a latency score of 180 sec. The latency to enter the dark chamber during this retention test session was used as the index of memory for the training experience. Inhibitory avoidance data were not distributed normally, so these results were evaluated using the nonparametric Mann-Whitney U test.

Elevated plus-maze. Anxiolytic-like activity was evaluated using the elevated plus-maze, a pharmacologically validated model (Brioni *et al.*, 1993; Pellow *et al.*, 1985) according to procedures previously described (Brioni *et al.*, 1993). The elevated plus-maze was custom made of gray Plexiglas and consisted of two open arms (17 × 8 cm) and two enclosed arms (17 × 8 × 15 cm) extending from a central platform (8 × 8 cm) mounted on a plywood base raised 39 cm above the floor. Light

levels on the open and enclosed arms were similar. A video camera was mounted on the ceiling above the apparatus and the experiments were taped for later behavioral evaluation. At the beginning of the experiment, mice were placed in the center of the maze and the following variables were scored: 1) the time spent in the open arms (a measure of anxiolytic-like activity) and 2) the number of entries into the four arms (a measure of general activity). An arm entry was defined as the entry of all four feet of the animal into one arm. The test lasted 5 min. All animals used were naive to the apparatus.

Body temperature, locomotor activity and rotorod performance. In these experiments, both body temperature and open field locomotor activity were measured in the same mice. Beginning 4 min after an i.p. injection of the nAChR ligands (ABT 418, (-)-nicotine or their enantiomers), horizontal activity counts were recorded for 15 min in a 41 × 41 cm. open field using Digiscan activity monitors (Omnitech Electronics, Columbus, Ohio). In some experiments, the nAChR channel blocker, mecamylamine, or saline was administered 4 min before the agonist. Body temperature was measured using a rectal probe inserted 3 cm into the rectum (YSI TeleThermometer, Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Body temperature was determined at two time points: approximately 20 min (immediately after the mice were removed from the open field) and 60 min after injection of the agonist. Temperature and locomotor activity data were analyzed using ANOVA (SuperANOVA, Abacus Concepts, Inc., Berkeley, CA), with post-hoc pairwise comparisons evaluated using Fisher's protected least significant difference test.

Motor coordination was assessed in mice using an accelerated rotorod test procedure using previously published methods (Jones and Roberts, 1967). Diazepam (3.5, 10.5 and 35.5 $\mu\text{mol/kg}$ i.p.) served as a CNS reference depressant.

Antinociceptive activity. The effects of ABT 418 on nociception were determined using the Woolfe-MacDonald hot-plate test (Woolfe and MacDonald, 1944). The latency to licking or shaking of the hind paw was measured after mice were placed on the hot plate (56.5°C) at 15, 30 and 60 min after dosing. Morphine sulphate (14.7 $\mu\text{mol/kg}$) served as a reference standard.

Seizure and anticonvulsant activity. Seizure liability was determined both in the presence and absence of the nicotinic receptor antagonist, mecamylamine. Five min after administration of mecamylamine (15 $\mu\text{mol/kg}$) or saline, (-)-nicotine or ABT 418 was administered i.p. and animals were observed for gross behavioral signs of seizure activity. Appropriate doses were selected on the basis of pilot experiments and seven animals were included in each group. Seizure values were calculated for each treatment condition using the method of Litchfield and Wilcoxon using the PHARM/PCS program (MicroComputer Specialists, Philadelphia, PA). Single doses with 100 and 0% seizures were used in the calculations of the ED_{50} values.

Anticonvulsant activity of ABT 418 was assessed by pretreating mice 30 min before the i.v. administration of pentylenetetrazol (0.5% solution infused at 0.3 ml/min). Diazepam (35.2 $\mu\text{mol/kg}$ i.p.) served as a reference compound.

Lethality. Lethality was assessed using a variation of the approximate lethal dose procedure. After initial range-finding experiments, approximate lethal dose values were determined in groups of seven mice, each mouse in a group receiving one of seven doses separated by 10- $\mu\text{mol/kg}$ increments. In each group of seven mice, the lowest dose at which an animal died within 24 hr of injection was designated as the approximate lethal dose for that group. Four such groups were used to determine the mean approximate lethal dose for ABT 418 and (-)-nicotine and three groups were used to determine the mean approximate lethal dose for A-81754.

Pharmacokinetic Analysis

ABT 418 and (-)-nicotine were extracted from rat plasma and brain using a mixture of 0.1 ml of biological tissue to 1.0 ml of buffer basified with 0.1 to 0.6 ml of 0.5 M K_2CO_3 . This mixture was extracted with 5 to 8 ml of hexane/ethyl acetate (1:1) by vortexing and centrifuging at 3,000 rpm for 15 min at 18°C. The organic phase was back extracted

with 0.3 ml of 0.02 N HCl by vortexing and centrifuging as indicated above. To prepare brain tissue homogenates the brain was homogenized in 5 × weight volume of cold 1 N perchloric acid and centrifuged at 18,500 rpm for 20 min at 4°C. The supernatant was adjusted to pH 10.6 with 2 M K_2CO_3 and treated as described above. Traces of organic solvent in the acid extract from plasma and brain was removed in a fume hood for 1.5 hr at room temperature without N_2 blowing or heating before analyses by high-performance liquid chromatography.

The analytical procedure consisted of injecting 5 to 50 μl of extract into a high performance liquid chromatographic system (Hewlett Packard model 1050, Naperville, IL) fitted with a C_{18} reverse phase column (15 × 0.46 cm internal diameter; ODS-AQ, 5- μm spherical particles, YMC) and a Coulchem II electrochemical detector (ESA, Bedford, MA). The electrochemical detector was fitted with a conditioning cell (model 5021) and an analytical cell (model 5010) interfaced with a Rainin integrator (Rainin Inc, Woburn, MA). Chromatography was accomplished isocratically at a flow rate of 1 ml/min using a mobile phase for ABT 418 consisting of acetonitrile, methanol and 50 mM K-PO_4 and 10 mM tetramethyl ammonium hydroxide (18:4:78), pH 6.8; whereas the mobile phase for (-)-nicotine consisted of acetonitrile, methanol and 20 mM K-PO_4 (10:10:80) with pH adjusted to 6.3 with tetramethyl ammonium hydroxide. Corresponding calibration curves were run and sample values calculated using the Rainin Dynamax program. The sensitivity of the method was 0.5 ng/ml in plasma and 1.0 ng/g in brain tissue.

Cerebral Blood Flow Measurements

Methods for surgical preparation of rats for electrical stimulation of brain and measurement of cerebral blood flow were described previously in detail (Linville and Arneric, 1991) and are summarized below. Studies were conducted on male Sprague-Dawley rats. Animals were anesthetized with urethane (1.5 g/kg) after induction with halothane (3.5% balance O_2) delivered through a nose mask. Thin-wall vinyl catheters (outer diameter, 0.03 inch) were placed in the left femoral vein and artery for drug administration and monitoring of cardiovascular parameters, respectively, and the trachea was cannulated. Animals were subsequently placed in a Kopf stereotaxic frame with the head positioned so that the floor of the IVth ventricle was horizontal (incisor bar position, -11 mm), ventilated at 80 cpm (Harvard Apparatus, model 680, Harvard Instrument Inc., South Natick, MA) with 100% O_2 and paralyzed with *d*-tubocurarine (0.6 mg/kg/hr i.m.).

The procedure for eliciting an increased cortical cerebral blood flow response requires the stereotaxic placement of a stainless steel concentric bipolar electrode into the basal forebrain. Cerebrovascular responsiveness, as measured by laser-Doppler flowmetry, was used to localize the most active site of the basal forebrain with 10-sec trains of 2-msec duration pulses, at a frequency of 50 Hz and intensity of 100 μA . Briefly, the laser-Doppler flowmetry probe (0.8 mm diameter) was stereotaxically positioned within a restricted cortical region (0.3 ± 0.3 mm anterior and 1.8 ± 0.5 mm lateral to Bregma). The laser-Doppler flowmetry monitor (model PF-3, Perimed, Stockholm, Sweden) does not display actual perfusion units. Therefore, for the experiments discussed, these values are treated as relative perfusion units and only used to determine the percentage of changes in cerebral blood flow.

Experimental procedure. When stimulus-induced cerebral blood flow increases of approximately 100% were repeatedly obtained in the absence of significant changes in arterial pressure (<10 mm Hg), and when the perfusion rate was stable in the absence of basal forebrain stimulation, the experimental testing was initiated. A frequency-response curve was generated at 12.5, 25 and 50 Hz, (100 μA constant current) stimulations. Subsequently, the animal was infused with the cholinergic agents i.v. and frequency-response curves using the identical stimulation parameters were generated during the 3-to-15-min postinjection period. Compounds were administered in order of increasing concentration, and the order of stimulus frequency presentation was counterbalanced between animals during the postinjection period so as not to bias frequency-graded responses by the time from administration of compound. For the experiments examining the effects of drugs on

the resting or basal forebrain-elicited cortical cerebral blood flow response the data were calculated as the percentage of changes followed by log-transformed before ANOVA, because comparing the percentage of changes across treatment groups cannot be assumed to follow a normal distribution. Two-way repeated measures ANOVA were used, both drug dose and stimulation frequency being within-subjects measures. Post-hoc analyses consisted of multiple paired comparisons (*t* tests) at each frequency using a Bonferroni correction of the statistical criterion dependent upon the number of comparisons made. The criterion of statistical analysis was $P < .05$.

EEG

Six-month-old male Wistar rats (Camm Research, Wayne, NJ) were surgically implanted with epidural recording electrodes according to methods described previously (Radek, 1993). The effects of (-)-nicotine or ABT-418 on the EEG of conscious, unrestrained rats were determined for recordings over the left parietal cortex (posterior, -2.0; lateral, 3.0 in mm from bregma). Amplified EEG signals (filtered at 1 and 100 Hz) at a digital sampling rate of 256 Hz were recorded using a computer-based acquisition and analysis system (Brainwave Systems, Bloomfield, CO). The EEG was evaluated by fast Fourier transform power analysis specific frequency bands. Two recordings were made from each rat, one after administration of saline and the other after administration of either (-)-nicotine or ABT 418 (1.9 $\mu\text{mol/kg}$ i.p. for each compound). The order of administration saline and the test compound was counterbalanced.

Emetic Liability

Emetic liability was assessed in male or female beagle dogs (10–20 kg). Conscious dogs were gently restrained and drug (500 nmol/kg dissolved in 10 ml of sterile saline) was slowly administered intravenously over a 2-min period. The presence or absence of emesis occurring over an 8-hr period was recorded.

Results

Mouse Behavioral Studies

Inhibitory (passive) avoidance. ABT 418 enhanced inhibitory avoidance retention test performance at a dose of 0.062 $\mu\text{mol/kg}$ (fig. 1; $P < .05$, two-tailed Mann-Whitney U test). Doses a log unit higher or lower than this effective dose did not significantly improve performance, resulting in the inverted U-shaped dose-response curve typically reported for memory-enhancing agents (Flood *et al.*, 1981; Gold, 1989). The effects

of ABT 418 and A-81754 were compared in a related experiment. ABT 418 was effective at 0.062 $\mu\text{mol/kg}$ ($P = .054$, two-tailed Mann-Whitney U test), whereas the enantiomer, A-81754, did not affect performance at this dose or at a dose 10 times higher (table 2). Pretreatment with mecamylamine, a nAChR channel blocker, at a dose which did not by itself affect performance (5 $\mu\text{mol/kg}$), prevented the memory-enhancing effect of ABT 418 (fig. 2).

Elevated plus-maze. ABT 418 (i.p.) induced a significant increase in the time spent in the open arms in mice after i.p. injections ($F_{(4,69)} = 2.9$; $P < .05$). The anxiolytic-like effect of ABT 418 was induced at the 0.19 and 0.62 $\mu\text{mol/kg}$ doses (fig. 3). In contrast, these doses of ABT 418 did not affect the general activity of the animals, as measured by total arm entries ($F_{(4,69)} = 0.3$; N.S., data not shown). The (*R*)-enantiomer of ABT 418, A-81754, did not produce an anxiolytic-like effect over the dose range (0.019–6.2 $\mu\text{mol/kg}$ i.p.) tested ($F_{(6,51)} = 1.26$; N.S., data not shown).

Body temperature and locomotor activity and rotarod performance. Body temperature and open field locomotor activity were both markedly reduced by (-)-nicotine (table 3). In contrast, (+)-nicotine had no apparent effect on body temperature and only a mild effect on locomotor activity (table 3). ABT 418 and A-81754 both reduced body temperature (table 3), although they were considerably less potent than (-)-nic-

TABLE 2

Comparison of the effects of ABT 418 and its (*R*)-enantiomer, A-81754, on inhibitory avoidance performance in mice
Values are mean \pm S.E.M.

Dose $\mu\text{mol/kg}$	n per Group	Latency to Cross sec
Saline	14	63.9 \pm 17.5
ABT 418		
0.019	15	68.5 \pm 17.8
0.062	9	114.1 \pm 11.9*
0.19	10	64.2 \pm 18.5
0.62	10	83.2 \pm 18.1
A-81754		
0.019	12	93.4 \pm 18.2
0.062	11	68.2 \pm 17.2
0.19	10	60.1 \pm 12.5
0.62	12	68.8 \pm 15.7

* Different from control ($P = 0.054$; two-tail Mann-Whitney U test).

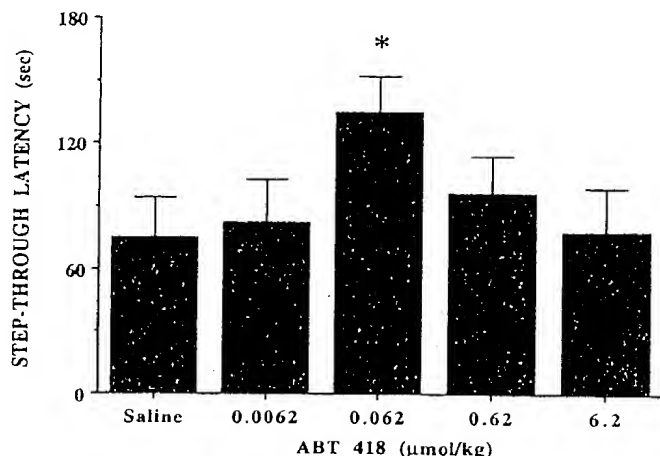


Fig. 1. Effect of ABT 418 on retention of inhibitory avoidance performance in mice. Values are mean \pm S.E.M.; $n = 12$. Different from saline; $P < .05$.

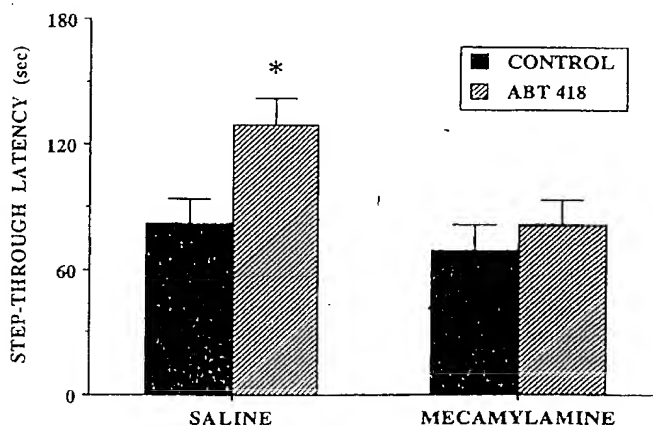


Fig. 2. Effect of pretreatment with mecamylamine (5 $\mu\text{mol/kg}$) on ABT 418-induced enhancement of inhibitory avoidance performance in mice. Values are mean \pm S.E.M.; $n = 27$ –28. Different from all other groups; $P < .02$.

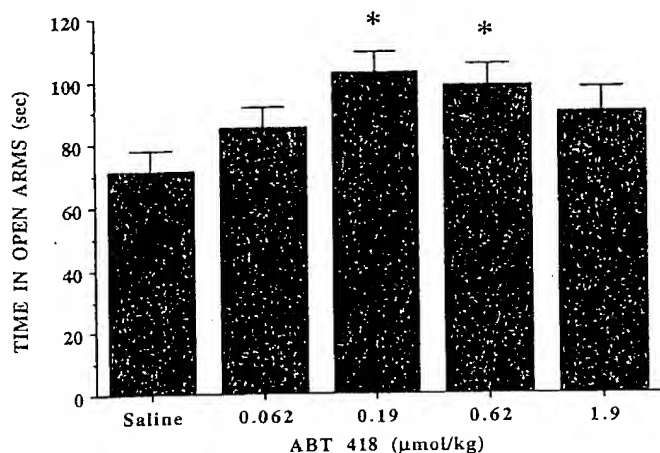


Fig. 3. Anxiolytic-like effect of ABT 418 in the mouse elevated plus-maze. Values are mean \pm S.E.M.; $n = 14$ to 16. *Different from saline; $P < .05$.

TABLE 3

Effect of (–)-nicotine, (+)-nicotine, ABT 418 and A-81754 on locomotor activity and body temperature

Values shown are the mean \pm S.E.M.; $n = 7$ to 8 per group. For the (–)- and (+)-nicotine activity data, the differences from saline control were evaluated by one-way ANOVA [$F_{(6,48)} = 12.57$; $P < .0001$] and Fisher PLSD post-hoc test (* $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$). For the ABT 418 and A-81754 activity data, the differences from saline control were evaluated by one-way ANOVA [$F_{(6,48)} = 3.53$; $P < .01$] and Fisher PLSD post-hoc test (* $P < .05$; ** $P < .01$). For (–)- and (+)-nicotine temperature data, the differences from saline control were evaluated by one-way ANOVA [$F_{(6,48)} = 125.92$; $P < .0001$ for 20 min and $F_{(6,48)} = 2.54$; $P < .05$ for 60 min] and Fisher PLSD post-hoc test (* $P < .05$; ** $P < .0001$). For the ABT 418 and A-81754 temperature data, the differences from saline control were evaluated by one-way ANOVA [$F_{(6,48)} = 11.52$; $P < .0001$ for 20 min and $F_{(6,48)} = 6.48$; $P < .0001$ for 60 min] and Fisher PLSD post-hoc test (* $P < .0001$).

Compound	Horizontal Activity Counts	Rectal Temperature at 20 min	Rectal Temperature at 60 min
$\mu\text{mol/kg}$			$^{\circ}\text{C}$
Saline	6131 \pm 345	37.0 \pm 0.1	37.1 \pm 0.1
(–)-Nicotine			
1.9	4274 \pm 741**	37.0 \pm 0.1	37.0 \pm 0.1
2	3655 \pm 615***	36.1 \pm 0.1**	36.7 \pm 0.3*
10	836 \pm 326****	34.5 \pm 0.2**	36.7 \pm 0.1*
(+)-Nicotine			
1.9	4554 \pm 273*	37.0 \pm 0.1	37.1 \pm 0.1
6.2	3838 \pm 542**	37.0 \pm 0.1	37.1 \pm 0.1
19.0	2608 \pm 223****	37.0 \pm 0.1	37.0 \pm 0.1
Saline	6028 \pm 606	37.2 \pm 0.04	37.1 \pm 0.04
ABT 418			
1.9	4475 \pm 484	37.1 \pm 0.05	37.1 \pm 0.06
6.2	4518 \pm 723	37.0 \pm 0.03	37.1 \pm 0.05
19.0	2643 \pm 509**	36.1 \pm 0.24*	36.8 \pm 0.14
A-81754			
1.9	4843 \pm 511	37.0 \pm 0.05	37.1 \pm 0.04
6.2	5306 \pm 169	37.1 \pm 0.05	37.0 \pm 0.03
19.0	4362 \pm 700*	36.4 \pm 0.19*	36.3 \pm 0.26*

otine. Interestingly, A-81754 appeared to have a longer-lasting effect on body temperature than either ABT 418 or (–)-nicotine, as it was the only compound tested that produced similar hypothermia at both the 20-min and 60-min time points. ABT 418 and A-81754 also decreased locomotor activity (table 3), although on this measure, ABT 418 was somewhat more potent than its enantiomer, A-81754. Neither compound was as potent nor as effective as (–)-nicotine in reducing locomotor activity, with ABT 418 producing a reduction in locomotor activity comparable to that produced by (+)-nicotine. Interestingly, the

potencies of ABT 418 and its enantiomer in producing reductions in body temperature and locomotor activity did not differ as much as did the relative potencies of the two nicotine enantiomers.

ABT 418 (0.62, 1.9 and 6.2 $\mu\text{mol/kg}$ i.p.) did not significantly affect the rotorod performance of mice at 15, 30 or 60 min after dosing (fig. 4). After a higher dose of ABT 418 (11.6 $\mu\text{mol/kg}$ i.p.), rotorod performance was significantly decreased at 15 min after dosing but returned to control values at 30 and 60 minutes after dosing. As expected, diazepam significantly decreased the rotorod performance of mice.

Antinociceptive activity. ABT 418 (0.62, 1.9 and 6.2 $\mu\text{mol/kg}$ i.p.) had no effect on paw-lick latency (data not shown). A dose of 19.0 $\mu\text{mol/kg}$ i.p., significantly elevated paw-lick latency at 30, but not at 15 or 60 min after dosing. This isolated antinociceptive effect was not considered a pharmacologically meaningful event. Morphine produced statistically significant increases in paw-lick latency at 15, 30 and 60 min after dosing.

Lethality. The approximate lethal dose for (–)-nicotine was 70 \pm 4 $\mu\text{mol/kg}$ i.p., which was approximately 2-fold lower than that seen with ABT 418 (137 \pm 5 $\mu\text{mol/kg}$). ABT 418, in turn, was lethal at a lower dose than its enantiomer, A-81754 (353 \pm 7 $\mu\text{mol/kg}$). Death occurred rapidly and was typically preceded by locomotor seizures.

Seizure and anticonvulsant activity. Seizures produced by ABT 418 and (–)-nicotine were similar in appearance. They typically occurred within a minute or two of injection and were preceded by a period of quiescence and labored breathing followed by a brief episode of uncontrolled, very rapid locomotor activity, "a running fit," and a period of tonic/clonic activity. The ED_{50} (95% confidence intervals shown in brackets) for seizures for ABT 418 was 61.6 $\mu\text{mol/kg}$ i.p. [50.7–74.8] which was somewhat higher than that for (–)-nicotine (40.8 $\mu\text{mol/kg}$ [33.9–49.2]), although the difference in potency of these two compounds to produce seizures was not as great as the difference in their lethal doses. Preadministration of the nAChR channel blocker, mecamylamine (15 $\mu\text{mol/kg}$ i.p.) substantially increased the seizure thresholds for both ABT 418 and (–)-nicotine, suggesting that the ABT 418-induced seizures are nicotine-mediated (mecamylamine + ABT 418 > 110 $\mu\text{mol/kg}$ i.p.; mecamylamine + (–)-nicotine = 69.4 [63.0–76.4]).

ABT 418 (0.62, 1.9, 6.2 and 19.0 $\mu\text{mol/kg}$ i.p.) had no effect on the latency to the onset of the three pentylenetetrazol-

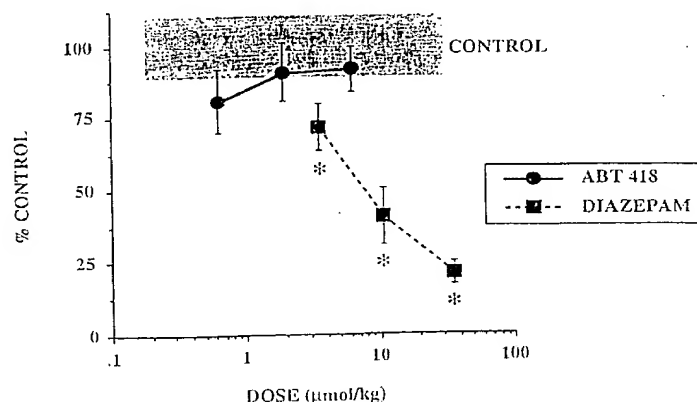


Fig. 4. ABT 418, in contrast to diazepam, does not impair rotorod performance in the anxiolytic dose range. Values are mean \pm S.E.M.; $n = 15$; shaded area represents control mean \pm S.E.M. *Different from control; $P < .05$.

induced seizure components: first-twitch, pseudoclonus and persistent convulsion (data not shown). A dose of 19.0 $\mu\text{mol/kg}$ i.p., produced a small, statistically significant shortening of the latency to death after persistent convulsions. As expected, diazepam produced statistically significant delays in the onset of all three seizure components and death.

Rat Pharmacokinetics

Fifteen min after i.v. administration of 1.0 $\mu\text{mol/kg}$ of ABT 418, plasma and brain levels of parent ABT 418 were nearly equivalent (plasma = 43 ± 4 ng/ml; brain = 50 ± 5 ng/g; $n = 3$). After administration of equivalent doses, plasma levels of (-)-nicotine were 50 ± 14 ng/ml ($n = 3$) similar to that seen with ABT 418. However, brain levels of (-)-nicotine were 282 ± 42 ng/g ($n = 3$), more than 5-fold greater than those seen in plasma. (-)-Nicotine also had twice the plasma half-life (42 ± 3 min; $n = 4$) of ABT 418 (23 ± 5 min; $n = 6$). In contrast, the oral bioavailability of (-)-nicotine ($16 \pm 5\%$; $n = 2$) was less than ABT 418 ($27 \pm 3\%$; $n = 3$).

Cerebral Blood Flow in Anesthetized Rat

At doses ranging from 0.002 to 2.0 $\mu\text{mol/kg}$ i.v., ABT 418 had no effect on resting cerebral blood flow (table 4). Further, no effects on mean arterial blood pressure were observed over this dose range (table 4).

Electrical microstimulation of the basal forebrain elicits frequency-graded (6.25–50 Hz) increases in cortical cerebral blood flow up to $173 \pm 8\%$ of resting control values ($n = 7$ –9) (table 4). These increases occurred in the absence of any observable change in mean arterial pressure on the original blood pressure trace (data not shown).

ABT 418, administered i.v., produced a 40% enhancement of the basal forebrain-elicited cortical cerebral blood flow response without significantly affecting resting cerebral blood flow (table 4). A significant increase was found at both 25 Hz ($180 \pm 10\%$ of resting control value; $n = 7$) and at 50 Hz ($202 \pm 5\%$ of resting control values; $n = 7$) after administration of 0.002 $\mu\text{mol/kg}$ of ABT 418. The enhancement was not observed at higher doses.

EEG in Conscious Rat

The doses chosen for ABT 418 and (-)-nicotine were based on doses of these agents that maximally improve septal lesion-induced deficits in water maze performance in rats (Decker et al., 1992, 1994). The effects of ABT 418 and (-)-nicotine on free-run EEG were assessed for 20 min after injection using a dose of 1.9 $\mu\text{mol/kg}$ i.p. for each compound (table 5). ABT 418

did not produce any significant effects in any of the frequency bands analyzed. In contrast, (-)-nicotine (1.9 $\mu\text{mol/kg}$ i.p.) significantly reduced total power in the 1 to 4 ($t = 5.4$; $P < .001$), 4 to 8 ($t = 5.9$; $P < .001$) and 8 to 13 Hz ($t = 4.7$; $P = .001$) bands and increased total power in the high frequency 25 to 50 Hz band ($t = -2.52$; $P = .036$). Thus, at equivalent doses known to enhance cognitive performance, these two nAChR ligands demonstrate a different profile of effects on free-run EEG.

Emetic Liability in Dog

(-)-Nicotine given in a dose of 500 nmol/kg i.v. elicited at least one bout of emesis in three of three dogs. In contrast, ABT 418 given at an equivalent dose did not produce emesis, or any other observable signs of gastrointestinal distress, in three of three dogs.

Discussion

ABT 418, a potent and selective neuronal nAChR ligand (Arneric et al., 1994), shows stereoselective cognition-enhancing activity and nonsedating, anxiolytic-like activity in preclinical tests. This compound also enhances basal forebrain function as measured by increased basal forebrain-elicited cortical cerebral blood flow without causing generalized activation of the cortical EEG. ABT 418 has an improved safety profile relative to (-)-nicotine. A summary of the *in vivo* findings for ABT 418 is shown in table 6. These data suggest that ABT 418 does not elicit the dose-limiting side effects typically observed with (-)-nicotine (Arneric and Williams, 1993). The favorable behavioral effects of ABT 418 may be the result of potent activation of selective subtypes of nAChR, thereby maintaining the cognitive-enhancing and anxiolytic-like properties of (-)-nicotine while displaying an improved side effect profile relative to (-)-nicotine. Thus, ABT 418 may represent the first of a new class of nAChR channel ligands that have the potential of becoming safe and effective CNS therapeutic agents.

The current results indicate that memory, as assessed by retention of inhibitory avoidance training in mice, is improved by ABT 418. Administration of ABT 418 to mice before inhibitory avoidance training improved performance on a retention test conducted 24 hr later. The dose-response curve for this effect had an "inverted-U" shape that is characteristic of many memory-enhancing agents (Flood et al., 1981; Gold, 1989). The 10-fold difference between the minimally effective dose for ABT 418 in the current experiment (0.062 $\mu\text{mol/kg}$) and that of a related experiment with (-)-nicotine (0.62 $\mu\text{mol/kg}$) using

TABLE 4

Effects of ABT 418 on resting cortical cerebral blood flow, basal forebrain-elicited increased in cortical cerebral blood flow and mean arterial pressure

Values are mean \pm S.E.M.; $n = 7$ to 9. All data are expressed as a percentage of resting control values.

Parameter	Control	Dose of ABT 418 ($\mu\text{mol/kg}$ i.v.)			
		0.002	0.02	0.2	2.0
Resting cerebral blood flow	100.0 \pm 1.8	116.4 \pm 6.8	100.0 \pm 8.9	100.5 \pm 8.8	103.8 \pm 10.0
Basal forebrain-elicited cerebral blood flow at 12.5 Hz	116.6 \pm 2.4	135.1 \pm 7.3	123.3 \pm 9.9	121.8 \pm 11.3	122.3 \pm 10.7
Basal forebrain-elicited cerebral blood flow at 25 Hz	144.4 \pm 9.0	179.7 \pm 9.8*	152.3 \pm 8.9	150.6 \pm 6.1	160.0 \pm 11.9
Basal forebrain-elicited cerebral blood flow at 50 Hz	173.5 \pm 7.7	202.3 \pm 5.4*	170.0 \pm 10.0	168.8 \pm 11.0	182.2 \pm 14.9
Mean arterial pressure	98.9 \pm 5.7	101.9 \pm 3.1	87.8 \pm 6.6	91.7 \pm 6.7	88.3 \pm 6.5

* $P < .05$ using ANOVA.

TABLE 5
Effects of ABT 418 and (-)-nicotine on free run EEG in rats
Values are mean \pm S.E.M.; $n = 8$ to 9.

	Dose $\mu\text{mol/kg i.p.}$	Power				
		Frequency Band (Hz)				
		1-4	4-8	8-13 \sim	13-25	25-50
Control				8.1 ± 1.2	7.6 ± 1.1	3.3 ± 0.6
ABT 418	1.9	14.9 ± 1.4	18.7 ± 2.4	7.5 ± 1.2	5.8 ± 0.8	3.6 ± 0.2
(-)-Nicotine	1.9	$10.5 \pm 1.8^*$	$12.9 \pm 2.6^*$	$4.3 \pm 0.7^*$	5.3 ± 0.6	$4.7 \pm 0.6^*$

* $P < .05$, paired Students t test from saline control.

TABLE 6
Overview of the *in vivo* pharmacological properties of ABT 418 and the (*R*)-enantiomer, A-81754, compared to (-)-nicotine
ED_{min} is defined as the minimum dose of the drug that elicited a statistically significant response. ND, not determined; NA, not applicable.

Assay Procedure	ABT 418 (<i>S</i> -form)	A-81754 (<i>R</i> -form)	(-)-Nicotine (<i>S</i> -form)
Mouse inhibitory avoidance (ED _{min} , $\mu\text{mole/kg i.p.}$)	0.062	>0.62	0.62 ^a
Mouse elevated plus-maze (ED _{min} , $\mu\text{mol/kg i.p.}$)	0.19	>6.2	0.62 ^b
Rat cerebral circulation enhancement of basal forebrain vasodilation (ED _{max} , $\mu\text{mol/kg i.v.}$)	0.002	ND	0.43 ^c
Toxicity ($\mu\text{mol/kg i.p.}$, mice)			
ALD	138 ± 5	353 ± 7	70 ± 4
Seizure (ED ₅₀ ; 95% confidence intervals)	62 [51-75]	ND	41 [34-49]
Hypothermia	19	19	6.2
Therapeutic index (inhibitory avoidance vs. ALD)	2225	NA	113
Emetic liability in dog (dogs responding to 500 nmol/kg i.v.)	0/3	ND	3/3
Farmacokinetics			
MAAT $t_{1/2}$	23 min	24 min	42 min
Bioavailability (p.o.)	27%	ND	16%

^a From Brioni and Arneric, 1993.

^b From Brioni *et al.*, 1993.

^c From Linville *et al.*, 1993.

identical conditions (Brioni and Arneric, 1993) strongly suggests that ABT 418 is more potent in improving retention of inhibitory avoidance training than (-)-nicotine. Although effects on nonassociative performance factors cannot be completely ruled out when drugs are administered before training as in the current set of experiments, it is unlikely that the improved performance found with ABT 418 resulted from a drug-induced increase in pain sensitivity because ABT 418 did not alter pain thresholds in the hot-plate test and did not alter shock sensitivity in pilot experiments (data not shown).

The enhanced retention test performance seen with ABT 418 was not observed with A-81754, the (*R*)-enantiomer of ABT 418, at doses up to 10 times the minimally effective dose of ABT 418, indicating that these effects are stereoselective. Furthermore, this effect of ABT 418 appears to be mediated by actions at nAChRs because mecamylamine, a nAChR channel blocker, completely prevented the effect of ABT 418. Cognitive-enhancing properties of ABT 418 are implicated by the im-

proved retention of inhibitory avoidance training observed in the current study. The inhibitory avoidance task can be regarded as an example of simple fear conditioning but it still clearly involves a strong memory component, and effects of pharmacological manipulations on this task are frequently consistent with effects on more complex measures of cognitive function. This latter generalization appears to be the case with ABT 418 as well, as preliminary reports suggest that ABT 418 improves delayed matching to sample performance in monkeys (J. J. Buccafusco, personal communication) and enhances spatial discrimination water maze learning in septal-lesioned rats (Decker *et al.*, 1994). ABT 418-induced memory enhancement *via* stimulation of nAChR would be consistent with a body of literature suggesting that nAChR agonists can improve cognitive performance in experimental animals (Haroutunian *et al.*, 1985; Elrod *et al.*, 1988; Levin *et al.*, 1990; Hodges *et al.*, 1992; Decker *et al.*, 1992) and in humans (Wesnes and Warburton, 1978, 1984; Rusted and Eaton-Williams, 1991).

ABT 418 has anxiolytic-like activity in the elevated plus-maze after systemic injection of 0.19 and 0.62 $\mu\text{mol/kg}$, doses that did not affect the general level of activity on the maze. This anxiolytic-like effect of ABT 418 is also stereoselective in that the (*R*)-enantiomer of ABT 418, A-81754, did not have anxiolytic-like activity. ABT 418 was 3 times more potent than (-)-nicotine and 15 times more potent than diazepam when compared across related experiments (Brioni *et al.*, 1993). In the present study, no effects on motor coordination were observed with ABT 418 in the dose range where anxiolytic activity occurred, which is in contrast to the effects observed with diazepam. Thus, contrary to the accepted notion that reduction of anxiety is associated with significant amnesia and motor incoordination (as in the case of diazepam, barbiturates and alcohol), activation of some nAChRs can produce anxiolytic-like effects without either sedation or memory impairment in rodents.

In addition to producing memory improvement and anxiolytic-like activity, ABT 418 enhanced the basal forebrain-elicited cortical cerebral blood flow response. The effects of ABT 418 on cerebral blood flow in rats were observed at lower doses than the effects of ABT 418 on plus-maze activity and retention of inhibitory avoidance in mice. In addition to the difference in the species used to assess these effects, it should also be noted that the blood flow experiments were conducted in anesthetized animals and the drug was injected *i.v.*, both of which could have affected the dose response relationship. When compared to recently reported studies examining the effect of (-)-nicotine cortical cerebral blood flow (Linville *et al.*, 1993), the results of the present study suggest that ABT 418 is approximately 200 times more potent than (-)-nicotine in this para-

digm. The difference in potency between ABT 418 and (-)-nicotine in this model does not appear to be due to differences in the access of these two compounds to the brain after systemic administration. Furthermore, unlike (-)-nicotine, ABT 418 did not alter resting cerebral blood flow even at 10 times the dose that significantly enhanced the basal forebrain-elicited cortical cerebral blood flow response. Thus, ABT 418 appears to demonstrate some additional selectivity in its cerebral blood flow-enhancing effects by increasing basal forebrain-stimulated cortical cerebral blood flow without having nonspecific vasodilatory properties. ABT 418 would potentially be selective in enhancing cerebral blood flow regulated by the cholinergic basal forebrain-cortical system which exhibits profound impairment in AD. The utility of compounds that activate nAChR channels to ameliorate cerebral blood flow impairments in specific disease conditions such as AD may warrant further consideration. It is assumed that the observed cerebral blood flow impairments in AD are symptomatic of this disorder and do not represent the causative or primary insult to the CNS. However, the role of the cholinergic basal forebrain in the regulation of cortical cerebral blood flow, and the degree of impairment of this system in AD, in particular the loss of cortical nAChR and of cortical cerebral blood flow, suggest that efforts to recover the loss in cortical cerebral blood flow by pharmacotherapeutic intervention may also contribute to cognitive benefits.

(-)-Nicotine is known to produce cortical arousal activity which is characterized by a low amplitude EEG (Yamamoto and Domino, 1965). This was confirmed and quantified by calculating power (in μV) of EEG signals using fast Fourier transform analysis in the present study. (-)-Nicotine produced cortical arousal at a dose previously shown to elicit cognitive enhancement (Decker *et al.*, 1992). Specifically, (-)-nicotine (1.9 $\mu\text{mol/kg}$ i.p.) significantly reduced power in the lower frequency bands (1–4, 4–8 and 8–13 Hz) and increased power in the higher frequency bands (25–50 Hz). In contrast, the same dose of ABT 418 did not affect EEG power values in any of the frequency bands examined. Thus, ABT 418 displays an EEG profile that can be differentiated from (-)-nicotine. This may be of potential clinical importance for an aged patient population that already exhibits significant sleep disturbances and abnormal EEG patterns. Anecdotal reports suggest that (-)-nicotine patches cause similar sleep disturbances which, with chronic use, may lead to a further exacerbation of the cognitive and affective decline. ABT 418 may not cause the additional disruption of sleep that would come from elevated levels of (-)-nicotine (Soldatos *et al.*, 1980).

Although doses of ABT 418 that have memory-enhancing, anxiolytic-like and cerebral blood flow-enhancing activities are lower than those required to obtain comparable effects with (-)-nicotine, ABT 418 is less potent than (-)-nicotine in producing acute toxicity. ABT 418 is significantly less potent than (-)-nicotine in producing overt seizure activity and death, although the separation between the potencies of these two compounds is somewhat less for seizure liability than for lethality. ABT 418-induced seizures appear to involve nAChRs because both (-)-nicotine- and ABT 418-elicited seizures were blocked by mecamylamine. Although high doses of ABT 418 did produce overt seizures, subeffective doses of the compound did not have proconvulsant actions when combined with pentylenetetrazol.

ABT 418 was also significantly less potent than (-)-nicotine in producing hypothermia and reducing locomotor activity.

Interestingly, A-81754, the (*R*)- enantiomer of ABT 418, was more toxic in these acute safety studies than would have been predicted from the binding studies. This enantiomer has a *K_i* value about 14 times higher than that for ABT 418 (Arneric *et al.*, 1994) but was only slightly less potent than ABT 418 in reducing temperature and activity and had a lethal dose only 2 to 3 times higher than that for ABT 418. This discrepancy could be related to actions by the enantiomer at other receptors or loss of stereoselectivity with high dose effects. Alternatively, A-81754 could be metabolized into a more toxic metabolite.

As previously discussed, clinical data suggest that (-)-nicotine can improve cognitive function in both normal people and AD patients. Similarly, (-)-nicotine has anxiolytic-like effects in stressful situations (Gilbert, 1979; Gilbert *et al.*, 1989). However, there are several key issues related to administering (-)-nicotine or other nicotine-like compounds to a predominantly non-smoking, aged patient population over the course of a chronic disorder such as AD. As Sunderland *et al.* (1988) have noted, in AD patients, alterations in mood and cardiovascular liabilities associated with high doses of (-)-nicotine would make this a difficult drug to use on a chronic basis. With respect to the cardiovascular system there is abundant evidence that (-)-nicotine activates the sympathetic nervous system both through central activation of sympathetic outflow and indirectly through the enhanced release of circulating catecholamines (Benowitz, 1992). These actions can result in elevations in blood pressure and elevated levels of lipids that would potentially predispose an individual to atherosclerosis, and an increased workload to the heart (Benowitz, 1992) and may also lead to acute myocardial infarction and sudden death. There are also gastrointestinal and addiction liabilities associated with (-)-nicotine. (-)-Nicotine patches given to non-smokers frequently cause nausea and complaints of gastrointestinal distress. Although addiction liability may be of concern to a non-AD patient population, AD patients who previously smoked appear to lose the drive to continue smoking (Barclay and Kheifets, 1988). However, it is clear that for maximal efficacy to be achieved, side-effect liabilities will have to be minimal. Although the improved safety profile of ABT 418 in rodents is not necessarily predictive of effects that might be found in humans, it is notable that early phase I safety testing in healthy young volunteers indicates that ABT 418 appears to be very well tolerated (Sebree *et al.*, 1993), and reduced side effect liabilities of ABT 418 would provide a significant advantage for this agent in the treatment of AD.

In summary, in rodents ABT 418 improves retention of inhibitory avoidance training, has anxiolytic-like effects as measured by the elevated plus-maze and enhances basal forebrain stimulation-induced cortical cerebral blood flow. ABT 418 is at least as potent as (-)-nicotine in producing all of these potentially beneficial effects but is significantly less potent than (-)-nicotine in producing several undesirable side effects in rodents. The unique pharmacological profile of this compound could represent a significant advantage for the safe and effective therapeutic approach for the amelioration of cognitive and emotional disturbances accompanying AD or other related CNS disorders.

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References

- ARAUJO, D. M., LAPCHAK, P. A., ROBITAILLE, Y., GAUTHIER, S. AND QUIRION, R.: Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. *J. Neurochem.* 50: 1914-1923, 1988.
- ARNERIC, S. P., SULLIVAN, J. P., BRIGGS, C. A., DONNELLY-ROBERTS, D., ANDERSON, D. J., RASZKIEWICZ, J. L., HUGHES, M. L., CADMAN, E. D., ADAMS, P., GARVEY, D. S., WASICAK, J. T. AND WILLIAMS, M.: (S)-3-Methyl-5-(1-Methyl-2-Pyrrolidinyl) Isoxazole (ABT 418): A Novel Cholinergic Ligand with Cognition-Enhancing and Anxiolytic Activities: I. *In Vitro* Characterization. *J. Pharmacol. Exp. Ther.* 270: 310-318, 1994.
- ARNERIC, S. P. AND WILLIAMS, M.: Nicotinic agonists in Alzheimer's disease: Does the molecular diversity of nicotine receptors offer the opportunity for developing CNS selective cholinergic channel activators? *In Recent Advances in the Treatment of Neurodegenerative Disorders and Cognitive Function*, Capri, Italy, 1993.
- AUBERT, I., ARAUJO, D. M., CECYRE, D., ROBITAILLE, Y., GAUTHIER, S. AND QUIRION, R.: Comparative alterations of nicotinic and muscarinic binding sites in Alzheimer's and Parkinson's diseases. *J. Neurochem.* 58: 529-541, 1992.
- BARCLAY, L. AND KHEYFETS, S.: Tobacco use in Alzheimer's disease. *Alzheimer's Disease and Related Disorders* 2: 172-178, 1988.
- BARTUS, R. T., DEAN, R. L., III, BEER, B. AND LIPPS, A. S.: The cholinergic hypothesis of geriatric memory dysfunction. *Science (Washington, DC)* 217: 78-417, 1982.
- BARTUS, R. T., DEAN, R. L., PONTECORVO, M. J. AND FLICKER, C.: The cholinergic hypothesis: A historical overview, current perspective, and future directions. *Ann. N. Y. Acad. Sci.* 444: 332-358, 1985.
- BENOWITZ, N. L.: Nicotine and coronary heart disease. *Trends Cardiovasc. Med.* 1: 315-321, 1992.
- BRIONI, J. D., ARNERIC, S. P.: Nicotinic receptor agonists facilitate retention of avoidance training: Participation of dopaminergic mechanisms. *Behav. Neural Biol.* 59: 57-62, 1993.
- BRIONI, J. D., O'NEILL, A., KIM, D. J. B. AND DECKER, M. W.: Nicotinic receptor agonists exhibit anxiolytic-like effects on the elevated plus-maze. *Eur. J. Pharmacol.* 238: 1-8, 1993.
- BUCCAFUSCO, J. J. AND JACKSON, W. J.: Beneficial effects of nicotine administered prior to a delayed matching-to-sample task in the young and aged monkeys. *Neurobiol. Aging* 12: 233-238, 1991.
- COYLE, J. T., PRICE, D. L. AND DELONG, M. R.: Alzheimer's disease: A disorder of cortical cholinergic innervation. *Science (Washington, DC)* 219: 1189-1190, 1983.
- DASTUR, D. K.: Cerebral blood flow and metabolism in normal human aging, pathological aging and senile dementia. *J. Cereb. Blood Flow Metab.* 5: 1-9, 1985.
- DECKER, M. W., BRIONI, J. D., BUCKLEY, M. J., CURZON, P., AND ARNERIC, S. P.: Preclinical cognitive effects of cholinergic channel ligands. Abstracts of the XIXth C. I. N. P. Congress, 1994, in press.
- DECKER, M. W. AND MAJCHRAK, M. J.: Effects of systemic and intracerebroventricular administration of mecamylamine, a nicotinic cholinergic antagonist, on spatial memory in rats. *Psychopharmacology* 107: 530-539, 1992.
- DECKER, M. W., MAJCHRAK, M. J. AND ANDERSON, D. J.: Effects of nicotine on spatial memory deficits in rats with septal lesions. *Brain Res.* 572: 281-285, 1992.
- DECKER, M. W., MAJCHRAK, M. J., AND ARNERIC, S. P.: Effects of lobeline, a nicotinic receptor agonist, on learning and memory. *Pharmacol. Biochem. Behav.* 45: 571-576, 1993.
- ELROD, K., BUCCAFUSCO, J. J. AND JACKSON, W. J.: Nicotine enhances delayed matching-to-sample performance in primates. *Life Sci.* 43: 277-287, 1988.
- FLOOD, J. F., LANDRY, D. W. AND JARVY, M. E.: Cholinergic receptor interactions and their effects on long-term memory processing. *Brain Res.* 215: 177-185, 1981.
- GARVEY, D. S., WASICAK, J., DECKER, M. W., BRIONI, J. D., SULLIVAN, J. P., CARRERA, G. M., HOLLADAY, M. H., ARNERIC, S. P. AND WILLIAMS, M.: Novel isoxazoles which interact with brain cholinergic channel receptors have intrinsic cognitive enhancing and anxiolytic activities. *J. Med. Chem.* 37: 1055-1059, 1994.
- GEANEY, D. P., SOPER, N., SHEPSTONE, B. J. AND COWEN, P. J.: Effect of central cholinergic stimulation on regional cerebral blood flow in Alzheimer disease. *Lancet* 335: 1484-1487, 1990.
- GILBERT, D. G.: Paradoxical tranquilizing and emotion-reducing effect of nicotine. *Psychol. Bull.* 86: 643-661, 1979.
- GILBERT, D. G., ROBINSON, J. H., CHAMBERLIN, C. L., SPIELBERGER, C. D.: Effects of smoking nicotine on anxiety, heart rate and lateralization of EEG during a stressful movie. *Psychophysiology* 26: 311-319, 1989.
- GITELMAN, D. R. AND PROHOVNIK, I.: Muscarinic and nicotinic contributions to cognitive function and cortical blood flow. *Neurobiol. Aging* 13: 313-318, 1992.
- GOLD, P. E.: Neurobiological features common to memory modulation by many treatments. *Animal Learning Behav.* 17: 94-100, 1989.
- HAMEL, E. AND ESTRADA, C.: Cholinergic innervation of pial and intracerebral blood vessels: Evidence, possible origins and sites of action. *In Neurotransmission and Cerebrovascular Function*, Vol. II, ed. by J. Seylaz and R. Sercombe, pp. 151-173, Elsevier Science Publishers, New York, 1989.
- HAROUTUNIAN, V., BARNES, E. AND DAVIS, K. L.: Cholinergic modulation of memory in rats. *Psychopharmacology* 87: 266-271, 1985.
- HEISS, W.-D., SZELIES, B., ADAMS, J., KESSLER, G., PAWLK, G. AND HERHOLZ, K.: PET scanning for the detection of Alzheimer's disease. *In Early Markers in Parkinson's and Alzheimer's Diseases (New Vistas in Drug Research, Vol. 1)*, ed. by P. Sostert, P. Riederer, M. Strolin-Benedetti and R. Roncucci, pp. 181-196, Springer-Verlag, Austria, 1990.
- HODGES, H., SINDEN, J., TURNER, J. J., NETTO, C. A., SOWINSKI, P. AND GRAY, J. A.: Nicotine as a tool to characterize the role of the basal forebrain cholinergic projection system in cognition. *In The Biology of Nicotine: Current Research Issues*, ed. by P. M. Lippello, A. C. Collins, J. A. Gray and J. H. Robinson, pp. 157-182, New York, Raven Press, 1992.
- JONES, B. J. AND ROBERTS, D. J.: The quantitative measurement of motor incoordination in naive mice using an accelerating rotarod. *J. Pharm. Pharmacol.* 20: 302-304, 1967.
- JONES, G. M. M., SAHAKIAN, B. J., LEVY, R., WARBURTON, D. M. AND GRAY, J. A.: Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. *Psychopharmacology* 108: 485-494, 1992.
- LEE, T. J.-F.: Cholinergic mechanism in the large cat cerebral artery. *Circ. Res.* 50: 870-879, 1982.
- LEVIN, E. D.: Nicotinic systems in cognitive function. *Psychopharmacology* 108: 417-431, 1992.
- LEVIN, E. D., CASTONGUAY, M. AND ELLISON, G. D.: Effects of the nicotinic receptor blocker, mecamylamine, on radial-arm maze performance in rats. *Behav. Neural Biol.* 48: 206-212, 1987.
- LEVIN, E. D., LEE, C., ROSE, J. E., REYES, A., ELLISON, G., JARAVIK, M. AND GRITZ, E.: Chronic nicotine and withdrawal effects on radial-arm performance in rats. *Behav. Neural Biol.* 53: 269-276, 1990.
- LINVILLE, D. G. AND ARNERIC, S. P.: Cortical cerebral blood flow governed by the basal forebrain: age-related impairments. *Neurobiol. Aging* 12: 503-510, 1991.
- LINVILLE, D. G., WILLIAMS, S., RASKIEWICZ, J. L. AND ARNERIC, S. P.: Nicotinic agonists modulate basal forebrain (BF) control of cortical cerebral blood flow in anesthetized rats. *J. Pharmacol. Exp. Ther.* 267: 440-448, 1993.
- MEYER, E. M., DE FIEBRE, C. M., HUNTER, B. E., SIMKINS, C. E., FRAUWORTH, N. AND DE FIEBRE, N. E. C.: Effects of anabaseine-related analogs on rat brain nicotinic receptor binding and on avoidance behaviors. *Drug Devel. Res.* 31: 127-134, 1994.
- NEWHOUSE, P. A., POTTER, A., CORWIN, J. AND LENOX, R.: Acute nicotinic blockade produces cognitive impairment in normal humans. *Psychopharmacology* 108: 480-484, 1992.
- NEWHOUSE, P. A., SUNDERLAND, T., TARIOT, P. N., BLUMHARDT, C. L., WEINGARTNER, H., MELLOW, A. AND MURPHY, D. L.: Intravenous nicotine in Alzheimer's disease: A pilot study. *Psychopharmacology* 95: 171-175, 1988.
- OLIVERIO, A.: Effects of mecamylamine on avoidance conditioning and maze learning of mice. *J. Pharmacol. Exp. Ther.* 154: 350-356, 1966.
- PELLOW, S., CHOPIN, P., FILE, S. E., BRILEY, M.: Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Meth.* 14: 149-167, 1985.
- PERRY, E. K., TOMLINSON, B. E., BLESSED, G., BERGMANN, K. AND GIBSON, P. H.: Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br. Med. J.* 2: 1457-1459, 1978.
- PETIT, D., LORRAIN, D., GAUTHIER, S. AND MONTPLAISIR, J.: Regional spectral analysis of the REM sleep EEG in mild to moderate Alzheimer's disease. *Neurobiol. Aging* 14: 141-146, 1993.
- PINARD, E.: Cholinergic innervation of cerebral blood vessels: Functional aspects. *In Neurotransmission and Cerebrovascular Function*, Vol. II, ed. by J. Seylaz and R. Sercombe, pp. 175-191, Elsevier Science Publishers, New York, 1989.
- PROHOVNIK, L., MAYEUX, R., SACKEIM, H. A., SMITH, G., STERN, Y. AND ALDERSON, P. O.: Cerebral perfusion as a diagnostic marker of early Alzheimer's disease. *Neurology* 38: 931-937, 1988.
- RADEK, R.: Effects of nicotine on cortical high voltage spindles in rats. *Brain Res.* 625: 23-28, 1993.
- RIEKKINEN, P., JR., SIRVIÖ, J., AALTONEN, M. AND RIEKKINEN, P.: The effects of concurrent manipulations of nicotinic and muscarinic receptors on spatial and passive avoidance learning. *Pharmacol. Biochem. Behav.* 37: 405-410, 1990.
- RISBERG, J., GUSTAFSON, L. AND BRUN, A.: High resolution regional cerebral blood flow measurements in Alzheimer's disease and other dementia disorders. *In Alzheimer's Disease. Epidemiology, Neuropathology, Neurochemistry, and Clinics (Key Topics in Brain Research)*, ed. by L. Maurer, P. Riederer and H. Beckmann, pp. 509-516, Springer-Verlag, New York, 1990.
- RUSTED, J. AND EATON-WILLIAMS, P.: Distinguishing between attentional and amnesic effects in information processing: The separate and combined effects of scopolamine and nicotine on verbal free recall. *Psychopharmacology* 104: 363-366, 1991.
- SAHAKIAN, B., JONES, G., LEVY, R., GRAY, J. AND WARBURTON, D.: The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of the Alzheimer type. *Br. J. Psychiatry* 154: 797-800, 1989.
- SCHRODER, H., GIACOBINI, E., STRUBLE, R. G., ZILLES, K. AND MAELICKE, A.: Nicotinic cholinergic neurons of the frontal cortex are reduced in Alzheimer's disease. *Neurobiol. Aging* 12: 259-262, 1991.
- SEBREE, T., GREBB, J., KITTLE, C., AND KASHKIN, K.: A phase I, single, rising-dose study of A-81418, a novel cholinergic channel activator (ChCA). *American College of Neuropsychopharmacology*, Hawaii, December, 1993.
- SHIMOHAMA, S., TANIGUCHI, T., FUJIWARA, M. AND KAMEYAMA, M.: Changes

- in nicotinic and muscarinic cholinergic receptors in Alzheimer-type dementia. *J. Neurochem.* 46: 288-293, 1986.
- SOLDATOS, C. R., KALES, J. D., SCHARF, M. B., BIXLER, E. O. AND KALES, A.: Cigarette smoking associated with sleep difficulty. *Science* (Washington, DC) 207: 551-553, 1980.
- SUNDERLAND, T., TARIOT, P. N. AND NEWHOUSE, P. A.: Differential responsivity of mood, behavior, and cognition to cholinergic agonists in elderly neuropsychiatric populations. *Brain Res. Rev.* 13: 371-389, 1988.
- TILSON, H. A., MCLAMB, R. L., SHAW, S., RODGERS, B. C., PEDIADITAKIS, P. AND COOK, L.: Radial-arm maze deficits produced by colchicine administered into the area of the nucleus basalis are ameliorated by cholinergic agents. *Brain Res.* 438: 83-94, 1988.
- WESNES, K. AND WARBURTON, D. M.: The effects of cigarette smoking and nicotine tablets upon human attention. In *Smoking Behavior: Physiological and Psychological Influences*, ed. by R. E. Thornton, pp. 131-147, Churchill-Livingston, London, 1978.
- WESNES, K. AND WARBURTON, D. M.: Effects of scopolamine and nicotine on human rapid information processing performance. *Psychopharmacology* 82: 147-150, 1984.
- WHITEHOUSE, P. J., MARTINO, A. M., ANTUONO, P. G., LOWENSTEIN, P. R., COYLE, J. T., PRICE, D. L. AND KELLAR, K. J.: Nicotinic acetylcholine binding sites in Alzheimer's disease. *Brain Res.* 371: 146-151, 1986.
- WHITEHOUSE, P. J., PRICE, D. L., CLARK, A. W., COYLE, J. T. AND DELONG, M. R.: Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.* 10: 122-126, 1981.
- WILLIAMS, M.: Tacrine - recommendation for approval. *Curr. Opin. Invest. Drugs* 2: 541-544, 1993.
- WOOLFE, G. AND MACDONALD, A. D.: The evaluation of the analgesic action of pethidine hydrochloride (Demerol). *J. Pharmacol. Exp. Ther.* 80: 300-307, 1944.
- YAMAMOTO, K. AND DOMINO, E. F.: Nicotine-induced EEG and behavioral arousal. *Int. J. Neuropharmacol.* 4: 359-373, 1965.

Send reprint requests to: Michael W. Decker, Neuroscience, D 47W, AP-10, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064-3500.

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